HARVEST PLUS PAPERS

Genotype by environment interaction effect on beta-carotene of yellow root cassava (*Manihot esculenta* Crantz) genotypes in Ghana

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Abstract

Nine yellow root and one white root cassava genotypes were evaluated in ten environments in Ghana for the variability of their beta carotene content in root. The aim was to identify cassava genotypes that have high beta carotene content in storage root to combat the widespread vitamin A deficiency for children under the age of five years and for pregnant and lactating women. This study was conducted in a Randomized Complete Bloc Design with nine yellow root genotypes namely 01/1224; 01/1235; 01/1368; 01/1371; 01/1412; 01/1417; 01/1442; 01/1610; 01/1663 and one white root cassava namely Wenchi009 as check. In 2005-2006 two experiments were conducted at Wenchi in the Forest-Savannah Transition zone and at Bunso in the Deciduous Forest zone and in 2006-2007, one additional location namely Pokuase in the costal savannah zone was added to Wenchi and Bunso to conduct the same field experiment. Each experiment was harvested two times (9 and 12 or 14 months after planting). At each harvest, beta carotene content analyses were carried out on yellow root cassava at the Nutrition Lab of Noguchi Medical Research Centre using High Performance Liquid Chromatography (HPLC) with a mobile phase made of acetonitrile: dichloromethane: methanol in the ratio 70:20:10 at a flow rate of 2.5 ml/min. Data collected were analyzed using the computer software GenStat Discovery Edition Release 4.2DE; MATMODEL 3.0; GGE biplot. Analyses of results showed statistically significant differences between genotypes for beta carotene content per root, beta carotene content in storage root per plant and but no difference for beta carotene concentration. The best genotype for beta carotene content was 01/1417 follow by 01/1371 and 01/1368.The differences between environments were highly significant for all beta carotene traits. The highest value of beta carotene concentration in fresh root was recorded in environments E_9, E_1, E_7 and E_5. These environments were all characterized by harvest at 9 months after planting. For beta carotene concentration, beta carotene content per storage root and beta carotene content in storage roots per plant the best environments were E_9 (9 MAP at Pokuase) and E_1 (9 MAP at Wenchi in 2005). The IMMI analysis has shown 01/1412 the most stable for beta carotene concentration. The highest average value of beta carotene content per storage root was registered for genotype 01/1253 followed by 01/1417 and 01/1412. The most stable genotype for beta carotene content per storage root was 01/1610 followed by 01/1371. The highest value was registered for genotype 01/1417 (also the most stable) followed by 01/1368 and 01/1235. Based on the above results the yellow root cassava genotypes 01/1368 and 01/1417 which combined high fresh storage root yield, high dry root yield with high beta carotene content in storage root and in storage root per plant were proposed for on farm testing and released to tackle the vitamin A deficiency in Ghana.

Keywords: Genotype, environment, yellow root cassava, beta carotene and heritability

Introduction

Vitamin A deficiency is a nutritional problem among many developing countries. In Sub-Saharan Africa, three million children under the age of five year suffer total or partial blindness caused by vitamin A deficiency (Hagenimana et al., 1999).

In Ghana vitamin A deficiency is a problem for children under the age of five years and for pregnant and lactating women. According to studies conducted by the Centre of Social Studies of the University of Ghana, vitamin A deficiency accounts for death of one out of six of all children between the ages of 6 and 59 months. Although these problems are enormous, their full magnitude is unappreciated because usually there are no obvious signs of the problems, and the victims themselves are not aware. As a result not enough attention is paid to vitamin A deficiency (Takyi Etor E. K. 1999). The primary source of all nutrients for people comes from agricultural products. Therefore, plant foods which provide concentrated pro-vitamin A carotenoids can

contribute to improved human health. Research has demonstrated that micronutrient-enrichment traits are available within the genomes of some major staple food crops including cassava, that could allow for substantial increases in the levels of pro-vitamin A carotenoids without negatively impacting crop yield (Welch, 2001). Studies have been conducted on retention of beta-carotene of cassava roots and have found that oven-drying, shadow drying and boiling retained the highest levels of beta-carotene (71.9%, 59.2% and 55.7%, respectively) and gari the lowest (about 34.1%) Chavez et al. 2007. Cassava is a major staple food in Ghana contributing 22% of Agricultural Gross Domestic Product (PPMED 1991) compared to 5% for maize, 2% for rice, sorghum and millet, 14% for cocoa, 11% for forestry, 7% for fisheries and 5% for livestock (Al-Hassan, 1989; Dapaah, 1996). Before it is utilized as food, the cassava storage root is almost invariably peeled. The peel comprises 10-20% of the storage root and of this the cork layer represents 0.5-2% of the total weight. The edible fleshy portion makes up 80-90% of the root. The storage root flesh is composed of about 62% water, 35% carbohydrate, 1-2% protein, 0.3% fat, 1-2% fibre and 1% mineral matter (IITA, 1982). Increasing the consumption of orange-fleshed cassava roots and their processed foods products can provide a significant proportion of the required dietary vitamin A intake.

There is the need, therefore, to evaluate some of the promising genotypes in different agro-ecological zones in Ghana to identify and select yellow root cassava varieties that are high yielding in terms of fresh storage root, dry matter content and high beta-carotene content.

The objectives of the study were to:

(i) Evaluate agronomic performance of yellow root cassava genotypes in three major agroecological zones of Ghana;
(ii) Evaluate beta carotene content in storage roots of yellow cassava genotypes grown in three major agroecological zones of Ghana;
(iii) Identify yellow root cassava genotypes that combine desirable agronomic traits (high fresh root yield and high dry matter content) with high beta carotene content in storage root.

Materials and Methods

The field experimentations of this study were conducted in three major agroecological zones of Ghana at three locations namely, Wenchi in the Forest-Savannah Transition agroecological zone, Bunso in the Deciduous Forest agroecological zone and Pokuase in the Coastal Savannah agroecological zone.

Combination of locations (Wenchi, Bunso and Pokuase); years (2005-2006 and 2006-2007) and harvest ages (9, and 12 or 14 months after planting MAP), gave a total of 10 different environments in which the experimentations were conducted. Descriptions of the 10 environments are presented in Table 1.

Table 1: Description of the 10 environments in which nine yellow root and one white root cassava genotypes were evaluated

Designation	Name of location	Year of experiment	Time of harvest
Envir onment 1			9 MAP
Environment 2		2005 -2006	14 MAP
Environment 3	Wenchi		9 MAP
Environment 4		2006 -2007	12 MAP
Environment 5			9 MAP
Environment 6		2005 -2006	14 MAP
Environment 7	Bunso		9 MAP
Environment 8		2006 -2007	12 MAP
Environment 9			9 MAP
Environment 10	Pokuase	2006 -2007	12MAP

From 38 cassava varieties introduced from IITA, nine yellow root cassava genotypes were selected based on the yellowish colour of the fresh storage root combined with their fresh root yield and their dry matter content. These genotypes together with one local white root cassava variety were established at two locations Wenchi and Bunso in July 2005, the experimentation was repeated at Wenchi, Bunso and Pokuase in July 2006.

The Randomized Complete Block Design (RCBD) as described by Gomez and Gomez (1984) was the experimental design used for all the five experimentations. At Wenchi and Pokuase, the lands were cleared and tilled with a disc plough to a depth of approximately 30cm. In Bunso, no tillage was used after spraying herbicide for land clearing. In each of the site the plot area was divided into three replications.

After each harvest, the beta carotene analysis was carried out in the Laboratory of de Department of Nutrition of the Noguchi Memorial

Institute, Legon. The beta-carotene analysis was carried out following the method of Rodriguez-Amaya and Kimura (2001)

Field and lab data collected was subjected to statistical analyses using GenStat Discovery Edition Release 4.2DE; MATMODEL 3.0, GGE biplot (Weikai Yan, 2006).

Results

Data were collected on many variables including fresh and dry root weight. But mainly the beta carotene related characteristics (beta carotene concentration, the beta carotene content per root, and beta carotene content per plant) were discussed in this paper.

The high average storage root yield per hectare (28.38 tons/ha) was obtained for genotype 01/1368 and the lowest was recorded by the local check Wenchi (8.49 tons/ha). The average yield among the 10 genotypes was 20.55 tons per hectare. There were highly significant differences ($P < 0.001$) among the genotypes, environments and genotype by environment interaction. Genotype, environment and genotype by environment interaction contributed 23.98%; 24.86% and 23.52% to the total sum of squares, respectively.

The average mean of dry matter content of the storage root was 31.50%. The analysis of variance revealed highly significant difference for storage root dry matter content among genotypes, environments and genotype by environment interaction. The highest dry matter (38.91%) was obtained for the local check Wenchi 009 followed by 01/1224 with 35.70%. The lowest dry matter (27.56%) was registered by the genotype 01/1371. The genotype, the environment and the G x E interaction for the storage root dry matter contributed 29.79%, 40.00% and 12.37% respectively to the total sum of squares.

Average fresh root yield and dry matter of ten yellow root cassava genotypes

Genotypes	Fresh roots yield per hectare (t/ha)	% root dry matter content	Root dry yield per ha (t/ha)
01/1224	17.37^{de}	35.70^{b}	6.16^{de}
01/1235	19.33^{cd}	29.27^{def}	5.39^{e}
01/1368	28.38^{a}	30.78^{d}	8.78^{a}
01/1371	14.35^{e}	27.56^{g}	3.99^{g}
01/1412	24.85^{abc}	29.47^{def}	7.05^{bcd}
01/1417	26.56^{ab}	29.00^{ef}	7.60^{abc}
01/1442	21.70^{bc}	28.62^{fg}	6.12^{de}
01/1610	19.41^{cd}	33.09^{c}	6.40^{cde}
01/1663	25.01^{abc}	32.57^{c}	8.05^{ab}
Wch009	8.49^{f}	38.91^{a}	3.26^{g}
Mean	*20.55*	*31.50*	*6.28*
Probability	< 0.001	< 0.001	< 0.001
LSD	3.908	1.682	1.275
CV	37.4%	10.5%	39.9%
s. e. d. G	1.98	1.96	0. 65

Beta-carotene concentration in fresh storage root (µg/g)

Average beta carotene concentration of 7 yellow root genotypes in 10 environments. The mean values of the beta carotene concentration ranged from 1.28 to 9.19 ìg/g fresh weight (Table 2). The combined analysis of variance showed no significant difference among genotypes and for G x E interaction. The difference among environments was significant (< 0.001). Based on the LSD 5%, environments were grouped into four categories. The highest beta carotene concentration was registered by a group of four environments E_9, E_1, E_7 and E_5 what corresponded all to nine months after planting. The genotype, the environment and the G x E interaction contributed respectively 3.17%, 26.88% and 23.07% to the total sum of squares (Table 3). The results of the AMMI analysis of the beta carotene concentration are shown in Table 4. From these results the first principal component axis (PCA 1) captured 51.21% of the interaction sum of squares in 25.92% of the interaction degrees of freedom. PCA 1 mean square was significant at $P < 0.05$.

Table 2. Average beta-carotene concentration in fresh storage root (ìg/g fresh weight) for seven yellow root cassava genotypes in 10 environments.

	Environments										
Genotypes	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}	Mean
01/1224	2.87	4.40	2.57	4.24	5.83	6.50	5.43	2.69	7.37	1.58	4.35
01/1235	7.67	3.23	1.99	3.52	4.42	3.23	8.94	2.07	5.26	2.05	4.24
01/1368	4.65	4.92	2.56	4.83	4.50	5.76	4.16	2.87	5.22	2.69	4.22
01/1371	5.47	5.65	2.82	4.87	5.50	5.41	4.90	3.57	7.69	3.19	4.91
01/1412	7.02	4.06	1.28	4.19	6.57	2.77	4.78	2.18	7.10	1.84	4.18
01/1417	6.13	4.49	2.61	4.15	5.52	4.51	9.19	2.45	4.27	2.93	4.63
01/1610	6.20	3.43	2.44	4.12	3.01	3.56	1.45	1.45	4.67	2.48	3.28
Mean	5.72^{ab}	4.31^{c}	2.32^{d}	4.28^{c}	5.05^{abc}	4.53^{bc}	5.55^{abc}	2.47^{d}	5.94^{a}	2.39^{d}	*4.26*
P value (Genotype)											0.17
P value (Environment)											< 0.001
LSD 5% (Genotype)											NS
LSD 5% (Environment)											1.36
P value (Genotype X Environment)											0.439
LSD 5% (Genotype X Environment)											NS
CV(%)											52.3

Table 3: Proportion of sum of squares for main effects and interaction for average beta carotene concentration in fresh storage root for seven yellow root cassava genotypes in 10 environments

Source of variation	DF	Mean squares	Contribution to SS
Genotype	6	0.065NS	3.17%
Environment	9	0.371***	26.88%
Genotype by environment	54	0.053NS	23.07%
Error	138	0.05	46.88%

*; **; ***: significant at 95%; 99% and 99.9%

Table 4: AMMI analysis of varianceincluding the first four interactions PCA axes for beta carotene concentration (mg/100g fresh weight) for seven yellow root cassava genotypes tested in 10 environments

Source	DF	SS	MS	Probability
Total	185	12.433	0.067	
Treatment	69	6.604	0.096	0.001**
Genotypes	6	0.395	0.066	0.258^{NS}
Environment	9	3.341	0.371	< 0.001***
G X E	54	2.868	0.053	0.395^{NS}
IPCA 1	14	1.469	0.105	0.017*
IPCA 2	12	0.669	0.056	0.359
IPCA 3	10	0.402	0.040	0.628
IPCA 4	8	0.215	0.027	0.828
Residual	10	0.113	0.011	0.993
Error	116	5.829	0.050	

*; **; ***: significant at 95%; 99% and 99.9%

Winning genotypes and mega-environment for beta-carotene concentration in fresh storage root (µg/g) based on GGE biplot

Figure 1 shows the mean performance and the stability of the genotypes for beta carotene concentration in fresh storage root. Genotype 01/1224 had the highest value followed by 01/1417 and 01/1371. Genotype 01/1610 had the lowest value. The most stable genotype was 01/1412.

Figure2 gives a polygon view of GGE biplot showing which genotypes won in which environments. The PC1 and PC2 together, which make up the GGE biplot, explained a total of 76.8% of the total variation. The vertex genotypes for the beta carotene concentration were 01/1235, 01/1610, 01/1224 and 01/1417. The genotypes 01/1371, 01/1412, and 01/1368 were located within the polygon and were found less responsive (Weikai et al., 2006). Environments E_2, E_3, E_4, E_5, E_6, E_8, E_9 and E_{10} fell in the sector with genotypes 01/1224, 01/1371 and 01/1368. Environment E_1 fell in the sector with genotype 01/1235. Environment E_7 fell in the sector with genotype 01/1417. No environment fell in the sectors with genotype 01/1610 as vertex genotype.

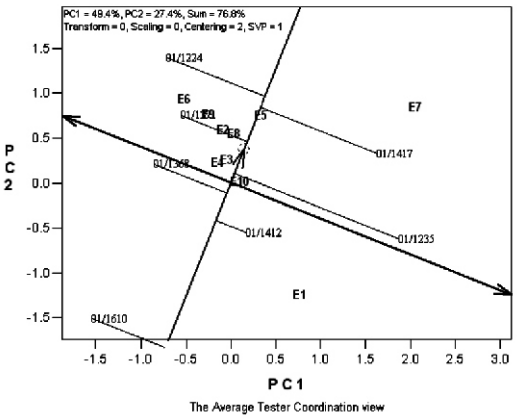

Figure 1: Mean performance and stability of seven yellow root cassava genotypes in 10 environments for beta carotene concentration in fresh storage root

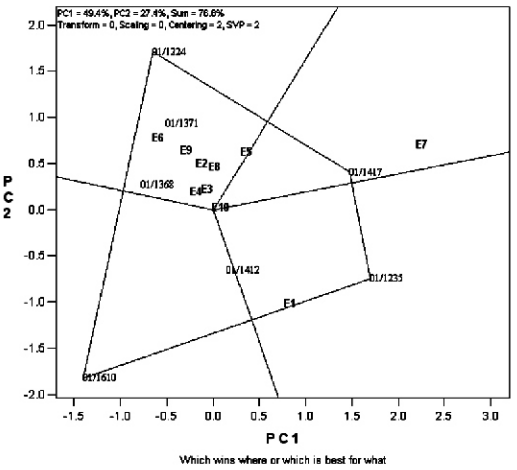

Figure 2: Mega-environment defined by different winning seven yellow root cassava genotypes tested in 10 environments for the beta carotene concentration in storage root.

Beta-carotene content per fresh storage root (mg)

Average beta-carotene content per fresh storage root (mg) of 7 yellow root genotypes in 10 environments

There was significant difference among genotypes (p=0.036) for beta carotene content per storage root. The difference between environments was also significant (P< 0.001) but there no significant difference for GXE interaction. The general mean of beta carotene content per storage root was 2.227 mg. Among the genotypes 01/1371 and 01/1417 recorded the highest beta carotene content per root.

The lower value (1.748 mg) was obtained with 01/1610. For the environments, the highest beta carotene content per storage root was obtained for E_2 (3.787 mg), E_6 (3.775 mg) and E_1 (3.033 mg). The environment contributed 38.74% to the total sum of squares while genotype and G x E interaction for 4.31% and 21.27%, respectively (Table 5). The AMMI analysis of the beta carotene content per storage root further showed that the first principal component axis (PCA 1) of the interaction captured 56.12% of the interaction sum of squares in less than 26% of the interaction degrees of freedom (Table 6). The mean squares for PCA 1 was significant at $P<0.01$.

Table 5: Average beta carotene content in individual fresh storage root for seven yellow root cassava genotypes in 10 environments.

	Environments										
Genotypes	*E*1	*E*2	*E*3	*E*4	*E*5	*E*6	*E*7	*E*8	*E*9	*E*10	Mean
01/1224	1.820	3.643	0.431	1.792	1.912	3.587	1.745	1.889	3.334	0.678	2.083 ab
01/1235	4.761	3.035	0.597	1.963	1.499	2.690	3.826	1.116	2.121	0.773	2.238 ab
01/1368	1.879	4.720	0.319	1.939	1.876	5.528	0.974	1.112	1.674	0.953	2.097 ab
01/1371	2.983	5.577	0.746	2.193	2.400	4.336	1.842	1.890	2.593	0.886	2.545 a
01/1412	3.438	2.802	0.183	2.887	2.915	3.194	1.548	1.25 2	4.528	1.268	2.402 ab
01/1417	3.562	2.925	0.580	2.346	2.922	3.794	3.570	1.980	1.647	1.451	2.478 a
01/1610	2.788	3.807	0.287	2.492	1.021	3.296	0.456	0.664	1.850	0.819	1.748 b
Mean	3.03 a	3.78 a	0.45 d	2.23 b	2.08 bc	3.77 a	1.99 bc	1.41 c	2.53 b	0.97 cd	*2.23*
P va lue (Genotype)											0.036
P value (Environment)											< 0.001
LSD 5% (Genotype)											0.67
LSD 5% (Environment)											0.801
P value (Genotype X Environment)											0.135
LSD 5% (Genotype X Environment)											NS
CV(%)											58.8

Table 6: Proportion of sum of squares for main effects and interaction for average beta carotene content in individual fresh storage root for seven yellow root cassava genotypes in 10 environments

Source of variation	DF	Mean squares	Contribution to SS
Genotype	6	4.15*	4.31%
Environment	9	24.87***	38.74%
Genotype by environment	54	2.27NS	21.27%
Error	138	1.77	35.68%

*; **; ***: significant at 95%; 99% and 99.9%

Table 7: AMMI analysis of variance including the first four interactions PCA axes for beta carotene content per storage root (mg) for seven yellow root cassava genotypes tested in 10 environments

Source	DF	SS	MS	Probability
Total	185	577.763	3.123	
Treatment	69	371.640	5.386	<0.001 ***
Genotypes	6	24.913	4.152	0.036 *
Environments	9	223.827	24.870	<0.001***
GXE	54	122.899	2.276	0.135^{NS}
IPCA 1	14	68.977	4.927	0.001 **
IPCA 2	12	22.818	1.902	0.392
IPCA 3	10	19.399	1.940	0.374
IPCA 4	8	7.116	0.889	0.854
Residual	10	4.588	0.459	0.989
Error	116	206.123	1.777	

^{NS} non significant; *; **; ***: significant at 95%; 99% and 99.9%

Winning genotypes and mega-environment for beta-carotene content per storage root (mg)

The PC1 and PC2 together, which make up the GGE biplots (Figures 3 and 4), explained a total of 72% of the total variation. The mean performance and the stability of the genotypes are shown in Figure 3. The highest average value was registered for genotype 01/1253 followed by 01/1417 and 01/1412. The most stable genotype was 01/1610 followed by 01/1371. Genotype 01/1368 had the lowest value followed by 01/1610 and 01/1371.. The highly unstable genotype was 01/1412 followed by 01/1235, and 01/1417.

Figure 4 gives a polygon view of GGE biplot showing which genotypes won in which environments. The vertex genotypes were 01/1235, 01/1417, 01/1368 and 01/1412. Two genotypes were found less responsive (01/1610 and01/1371) and were located within the polygon indicating that none of these two genotypes were best in any of the test environments. Three mega environments were defined. The first was the genotypes 01/1235 and 01/1417 winning-niche made of E_1, E_3, E_7, E_8, and E_{10}. The second mega environment fell in the sector with genotypes 01/1412 and 01/1224 made of environments E_4, E_5 and E_9. Environments E_2 and E_6 constituted the third mega environment with genotype 01/1368, 01/1610 and 01/1371.

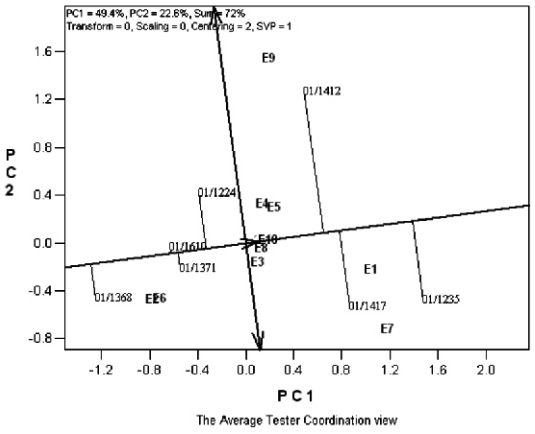

Figure 3: Mean performance and stability of seven yellow root cassava genotypes in 10 environments for beta carotene content per storage root

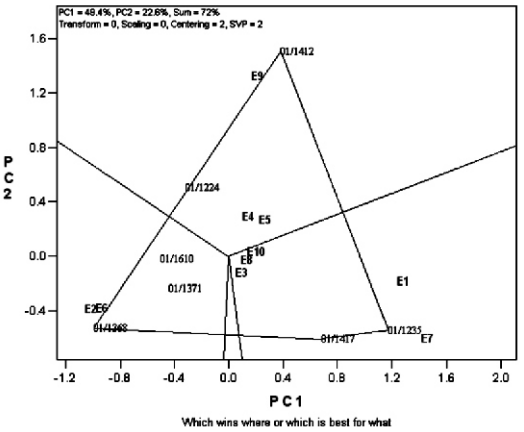

Figure 4: Mega-environment defined by different winning seven yellow root cassava genotypes tested in 10 environments for the beta carotene content per storage root.

Beta-carotene content in storage roots per plant (mg)

Average beta carotene content in storage roots per plants of 7 yellow root genotypes in 10 environments

The data of beta carotene content in storage root per plant ranged from 0.92 mg up to 29.27 mg and the general mean was 12.17 mg. Difference among genotypes was significant ($p= 0.029$) and the best genotypes for this variable were 01/1417 (14.25 mg) and 01/1368 (14.11 mg). The effect of the interaction G x E was not significant. There was significant difference ($p< 0.001$) among environments and any of the following four environments can be considered as best for this variable E_6 (20.25mg); E_2 (18.25 mg); E_9 (18.09 mg) and E_1 (17.29 mg). The G x E interaction has contributed 24.14% to the total sum of squares, while environment and genotype contributed respectively 34.14% and 4.68%, (Table 9). Table 10 shows the results of the AMMI analysis of the beta carotene content in storage roots per plant. These results showed that the first principal component axis (PCA 1) of the interaction captured 61.07% of the interaction sum of squares in 25.92% of the interaction degrees of freedom. Among the four PCAs only PCA 1 presented highly significant mean squares.

Table 8: Average beta carotene in fresh storage roots per plant (mg) for seven yellow root cassava genotypes in 10 environments

	Environments										
Genotypes	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	E_9	E_{10}	Mean
01/1224	12.29	16.42	1.42	8.35	7.55	12.63	8.66	8.92	23.38	3.88	10.35 ^{ab}
01/1235	21.17	14.06	4.08	15.37	5.94	11.13	16.42	6.24	18.21	6.30	11.89 ^{ab}
01/1368	13.61	22.37	3.05	13.44	12.98	38.10	9.79	7.44	13.41	6.89	14.11 ^{a}
01/1371	14.51	25.52	1.78	10.59	10.52	20.04	7.69	8.77	16.52	5.01	12.10 ^{ab}
01/1412	26.81	15.40	0.92	11.67	9.93	15.00	5.54	5.58	29.27	6.45	12.66 ^{ab}
01/1417	19.10	15.38	2.97	12.94	14.65	26.93	18.01	9.59	12.66	10.26	14.25 ^{a}
01/1610	13.57	18.57	1.77	16.46	4.41	17.95	2.60	3.22	12.98	7.19	9.87 ^{b}
Mean	17.29^{a}	18.25^{a}	2.23^{d}	12.69^{b}	9.82^{bc}	20.25^{a}	9.82^{bc}	7.11^{cd}	18.06^{a}	6.57^{cd}	*12.17*
P value (Genotype)											0.029
P value (Environment)											< 0.001
LSD 5% (Genotype)											4.218
LSD 5% (Environment)											5.041
P value (Genotype X Environment)											0.067
LSD 5% (Genotype X Environment)											NS
CV (%)											67.7

Table 9: Proportion of Sum of Squares for main effects and interaction for average beta carotene content in fresh storage root per plant of seven genotypes in 10 environments in Ghana

Source of variation	DF	Mean squares	Contribution to SS
Genotype	6	158.26*	4.68%
Environment	9	769.38***	34.14%
Genotype by environment	54	90.67^{NS}	24.14%
Error	138	64.74	37.03%

Table 10: AMMI analysis of variance including the first four interactions PCA axes for beta carotene content per plant (mg) of seven yellow-fleshed genotypes tested in 10 environments in Ghana

Source	DF	SS	MS	Probability
Total	185	20280.695	109.625	
Treatment	69	12770.552	185.080	< 0.001 ***
Genotypes	6	949.575	158.263	0.029 *
Environments	9	6924.468	769.385	0.001<***
G X E	54	4896.508	90.676	0.067^{NS}
IPCA 1	14	2990.285	213.592	< 0.001 ***
IPCA 2	12	710.454	59.205	0.535
IPCA 3	10	570.795	57.079	0.552
IPCA 4	8	407.831	50.979	0.615
Residual	10	217.144	21.714	0.969
Error	116	7510.144	64.743	

^{NS}non significant; *; **; ***: significant at 95%; 99% and 99.9%

Winning genotypes and mega-environment for beta-carotene content in roots per plant (mg) based on GGE biplot

Figure 5 shows the mean performance and the stability. Genotype 01/1224 had the lowest value followed by 01/1610, 01/1412 and 01/1371. The highest value was registered for genotype 01/1417 which was also the most stable; followed by 01/1368 and 01/1235. For this analysis the PC1 and PC2 together explained up to 73.7% of the total variation. The biplot of Figure 6 gives a polygon view of GGE biplot showing which genotypes won in which environments for beta-carotene content per plant. The vertex genotypes were 01/1412; 01/1235; 01/1417, 01/1368, 01/1610 and 01/1224. According to the Figure 6 four mega environments were defined. The first mega environment was the genotype 01/1417 winning-niche made of E_3, E_4, E_5, E_7, E_8, and E_{10}. The second fell in the sector with genotypes 01/1412 made of environment E_9. The third mega environment was the winning niche of genotype 01/1235 and made of environment E_1. Environments E_2 and E_6 constituted the fourth mega environment with genotype 01/1368. No environment fell in the sectors with genotype 01/1224 with genotypes 01/1610 and 01/1371.

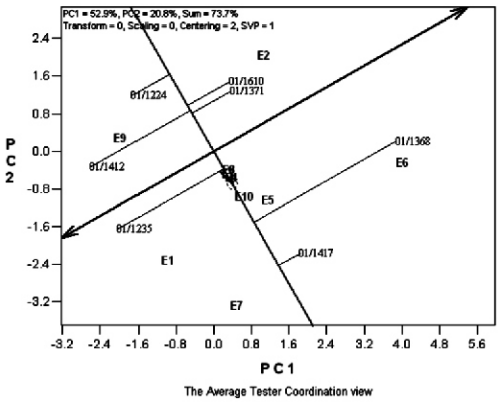

Figure 5: Mean performance and stability of seven yellow root cassava genotypes in 10 environments for beta carotene content in storage root per plant

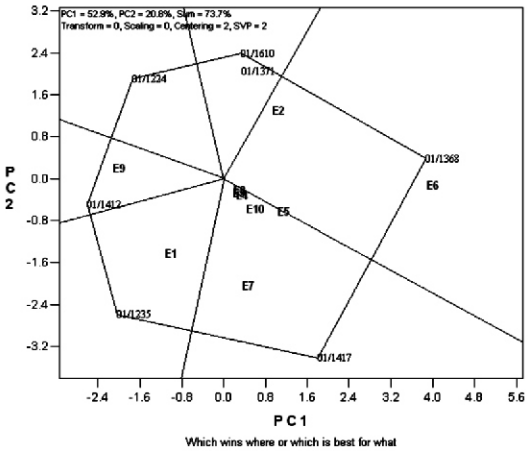

Figure 6: Mega-environment defined by different winning seven yellow root cassava genotypes tested in 10 environments for the beta carotene content in storage root per plant.

Correlation among the variables

Table 11 shows the correlations among the beta carotene and some agronomic traits for 7 yellow root genotypes. In general correlation between agronomic variables and beta carotene variables were highly significant except for beta carotene concentration. There was a highly significant correlation between beta carotene concentration and harvest index (Table 11). Beta carotene concentration was highly significant and positively correlated with harvest index only.

Table 11: Pearson product-moment correlations among beta carotene traits and agronomic variables for seven yellow root cassava genotypes tested in 10 environments in Ghana

	Beta Carotene content per plant	Beta Carotene content per root	Beta Carotene Concentration
Beta Carotene content per plant	1.000		
Beta Carotene co ntent per root	0.881***	1.000	
Beta Carotene Concentration	0.686***	0.757***	1.000
Mealiness	0.013 NS	0.008 NS	- 0.061 NS
Dry Matter	0.007 NS	0.042 NS	0.073 NS
Dry Yield	0.548***	0.342***	0.093 NS
Harvest Index	0.399***	0.444***	0.253***
Number of plant per hectare	0.165*	0.261***	- 0.048 NS
Number of root per hectare	0.073 NS	0.212** -	- 0.032 NS
Number of root per plant	0.293***	0.073 NS	- 0.013 NS
Dry root weight per plant	0.566***	0.420***	0.034 NS
Root weight	0.432***	0.507***	0.028 NS
Root weight per plant	0.579***	0.419***	0.021 NS
Top weight per hectare	0.110 NS	0.088 NS	- 0.102 NS
Top weight per plant	0.248***	0.063 NS	- 0.140 NS
Fresh root Yield	0.500***	0.0291***	0.057 NS

Discussion

Fresh storage roots yield is a trait with high G x E interaction effect (Mba and Dixon, 1995). This was observed in the present study emphasizing the importance of multi environmental evaluations of newly developed varieties to identify the ones best suited for different agroecologies. The average fresh storage root yield recorded in this work ranged from 8.49 to 28.38 t/ha with a grand mean of 20.55 ± 1.98 t/ha. These results are comparable to the range of 9.9 to 30.1 t/ha with a grand mean of 19.2 t/ha reported by IITA (1987) for a yield trial of 13 yellow root cassava genotypes harvested at 12 months in Ibadan, Nigeria. The range of the mean yield of this study is considered slightly better when compared to the fresh root mean yield range of 10.0 to 26.9 t/ha with a grand mean of 17.32 t/ha reported by Ssemakula and Dixon (2007) for 25 yellow cassava clones and three white-fleshed cassava (checks) at five locations in Nigeria for two years and harvested at 12 months after planting. This study's findings in terms of average yields are also similar to the range of 11.47 to 25.14 t/ha with a grand mean of 18.17 t/ha which were obtained by Maroya and Dixon (1992) for 10 white root cassava clones evaluated in four locations in Benin from 1989 to 1991.

Mahungu, (1998) reported that there is a shift in the paradigm factor and root yield alone is not sufficient to justify the production of a particular cassava variety. Root dry matter content is a critical factor among others. Braima *et al.,* 2000 stated that cassava varieties with 30% and above are said to have high dry matter content. In this study four of the nine yellow root cassava genotypes (01/1224, 01/1368, 01/1610 and

01/1663) had high dry matter content. However the white root cassava genotype used as check (Wenchi 009) had a dry matter content highly ($P<0.001$) superior to those of all the nine yellow root cassava genotypes. This finding confirmed the fact that yellow root cassava genotypes were considered to be characterized by relatively low dry matter (IITA, 1987). The average percentage root dry matter content recorded in this work ranged from 27.56 to 38.91% with a grand mean of 31.5%. These results are higher than the range of 25.0% to 34.7% with a grand mean of 29.17% reported by Ssemakula and Dixon (2007). These results are also better than the range between 23.7% and 33.1% with a grand mean of 28.82 reported by IITA 1987.

There was no difference in carotene concentrations among the seven yellow root cassava genotypes. It can be explained by the fact that during the preliminary visual evaluations only highly coloured flesh roots clones were selected. Values for the beta carotene concentrations obtained in this study ranged from 1.28 mg/kg to 9.19 mg/kg and were higher than the carotene values of 1.0 to 11.3 mg kg^{-1} dry weight from six cassava cultivars equivalent to about 0.3 to 3.8 mg kg^{-1} fresh weight as reported by McDowell and Oduro (1983), using the same HPLC method. Safo-Kantanka *et al.* (1984) estimated beta carotene content of cultivar BB (Banchi Bodea) to be about 3.2 mg per kg fresh weight in Ghana.

Ssemakula and Dixon (2007) also reported an overall mean value of 5.04 µg/g fresh weight for total carotenoid concentration for 25 yellow root cassava genotypes of the same families. A value of 5.07 µg/g fresh weight was also reported by CIAT 2005 for total carotenoid concentration in yellow cassava. These values are however comparable to 4.26 µg/g fresh weight for the present work that was calculated for only beta carotene and not total carotenoid. Similar studies in CIAT in 2005 gave a value of 4.07 µg/g for beta carotene which was really closed to 4.26 µg/g for this present study.

Conclusions

For beta carotene concentration the difference among environments was highly significant and the highest value of beta carotene concentration in fresh root was recorded in environments E_9, E_1, E_7 and E_5. All these four environments with the highest beta carotene concentration were characterized by harvest at 9 months after planting. Genotype 01/1412 was the most stable for beta carotene concentration but the highest value of beta carotene concentration was recorded for genotype 01/1224 which was also a vertex genotype for a mega-environment composed of eight environments including E_2, E_3, E_4, E_5, E_6, E_8, E_9 and E_{10}. The genotype 01/1224 which was a low yielding material can be used as female parental line in crossing blocks together with other high yielding yellow genotypes.

Significant differences were recorded for beta carotene content per fresh storage root and in storage roots per plant between genotypes ($p<0.05$) and between environments ($P< 0.001$). The highest value of beta carotene content per storage root was recorded for genotypes 01/1371 and 01/1417 but the genotype 01/1610 was the most stable for beta carotene content per storage root.

The highest value of beta carotene content in storage roots per plant was registered genotype 01/1417 which was also the most stable for the same. No significant difference was detected for Genotype by Environment Interaction for beta carotene content per fresh storage root and beta carotene content in storage roots per plant.

Significant differences ($P<0.05$) were found among genotypes for the beta carotene content in storage roots per hectare. Genotype 01/1368 has registered the highest value of beta carotene content in storage roots per hectare for which it was also a vertex genotype winning a mega-environment made of E_3, E_4, E_5, E_6, E_7, E_8 and E_{10}.

The genotype 01/1368 was followed by 01/1417 and the two combined high fresh and dry storage root yield with high beta carotene content together with 01/1412 can be immediately proposed as high yielding beta-carotene enriched cassava varieties for a nutritional programme to complement food for vitamin A deficiency.

Positive and highly significant correlations were found between some agronomic variables and beta carotene variables except beta carotene concentration which was only highly correlated with harvest index.

On the bases of the high agronomic performance characteristics such as number of plant harvested per hectare, fresh root yield per hectare, dry matter content, dry storage root yield per hectare etc., combined with beta carotene content characteristics, the genotypes 01/1368; 01/1412 and 01/1417; should be recommended for on-farm testing and if successful, should be proposed for released at least to be used for beta carotene enriched gari since their fresh and dry storage root yields and beta carotene content were high and stable.

For industrial use, it was proposed the genotype 01/1368 with highest performance in fresh root yield per hectare and highest beta carotene content per hectare.

For breeders who would like to improved beta carotene content of the local mealy genotypes through crossing it was proposed to consider the genotypes 01/1224; 01/1417 and 01/1368 as parental lines because of respectively (i) the high performance in beta carotene concentration in a very wide range of environments, (ii) high yield and the stability in beta carotene content per plant and (iii) the highest performance in fresh root yield and carotene content per hectare.

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Development of Molecular Marker for pro-vitamin A Carotenoids in Cassava

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Abstract
Cassava is currently the third most important source of calories in the tropics and consumed as a staple food. Several cassava varieties have yellow flesh color, and contain moderate amounts of carotenoid or beta-carotene which is a precursor of vitamin A. Consumption of carotene rich foods is the most effective intervention for vitamin A deficiency. The present work is envisaged towards the genetic improvement of carotenoid content (beta-carotene) in cassava, by identifying single nucleotide polymorphism (SNP) attributed to variation in carotenoid concentration among various cassava genotypes. phytoene synthase (PSY1), β-carotene hydroxylase (HYD1), lycopene β and ε cyclase (LYCB and LYCE), have been found to play a role in increasing levels of beta-carotene in plants. A total of 32 lines were drawn from the Uniform Yield Trial (UYT) stage of the cassava breeding program of IITA. The panel consists of lines with high carotenoid concentration (6ug-15ug) and low carotenoid concentration (0ug-5ug) as well as white root advanced clones. Primers for HYD1, LYCB, LYCE, and PSY1 genes designed from cassava ESTs were used to genotype the panel. Amplified PCR products were purified and sequenced. A total of 228 sequences were generated across all genes (HYD1, 49; LYCB, 62; PSY1, 59; LYCE, 58). Similarity search results of the sequences against NCBI database showed homology with pVAC genes in *Ricinus communis*, *Zea mays*, *Arabidopsis thaliana*, *Carica papaya*, and *Daucus carota* among others. Comparison of these sequences after multiple sequence alignment also revealed regions of conserved histidine cluster motifs that contain histidine residues: HXXX(X)H, HXX(X)HH, and HXXHH, characteristic domain of the β-carotene hydroxylase superfamily. Similarity search in Phytozome cassava (www.phytozome.net/cassava) matched full gene sequences of the carotenoid genes from which primers were designed. These primers were used

to genotype a larger panel of 40 (previous 32 inclusive) carotenoid variable lines. Upon sequence analysis, a nucleotide variant was discovered in a clone with high total carotene content. Discovered nucleotide variants will be validated and ultimately converted into user friendly assay for marker-assisted breeding. This approach will contribute to enhanced genetic improvement of nutritional quality of cassava roots and provides an insight into the genetic and molecular basis of carotenoid biosynthesis.

Keywords: Pro-vitamin A, cassava, phytoene synthase, lycopene, β-carotene hydroxylase, single nucleotide polymorphism.

Introduction

Cassava (Manihot esculenta crantz, Euphorbiaceae) is considered among third most important staple crop, also rich in calories, for one billion people in Africa, Asia and Latin America. Cassava production extends from West Africa belt and the adjoining Congo Basin, Sierra Leone, Nigeria, Tanzania, tropical South America to South East Asia. World production of cassava root was estimated to be 226 million tons in 2006, majority of production being from Africa (99.1 million tons), with Nigeria (33 million tons) making it the world largest producer of cassava. Cassava serves as food security crop to resource-poor farmers in developing countries like sub-Saharan Africa, and also a famine reserve (Stone, 2002).

Nutritionally, cassava tubers are rich in carbohydrates, but deficient in many proteins and many essential micronutrients. Tuber flesh color and good culinary quality are important traits for consumption of cassava as staple food. In most of the cultivated cassava the tuber flesh is white or cream which contain negligible amount of carotenoids (Bradbury and Holloway, 1988). The flesh color of cassava is associated with presence of carotenoids and the nutritive importance of carotenoids is attributed to its conversion to vitamin A as it is in the case of beta-carotene, and to its anti-oxidant property and ability to quench single oxygen in the case of lycopene. However, several cassava varieties with yellow flesh color contain moderate amounts of carotenoid or Beta-carotene which is a precursor of vitamin A (Mc Dowell, and Oduro, 1983), but yellow pigmented cassava is limited in cultivation in Africa (Oduro, 1981).

The devastating effects of vitamin A deficiency is a serious public health issue, attributed to over 4-6% of the disease burden in Sub-Saharan Africa (FAO, 2008) in many parts of sub-Saharan Africa, causing eye damage and blindness. Vitamin A is produced in the human body from its precursor Beta-carotene derived from plant sources, and 60% of the dietary vitamin A is estimated to come from pro vitamin A or beta-carotene, which is an antioxidant that helps prevent heart attacks or cancer, lowers cataract risks and macular disorders, and enhance the immune system. Cassava being a major staple food crop in sub-Saharan Africa, with an average consumption per capita as high as 400 grams/capita/day, and an average baseline of pro-vitamin A content as low as 0.5ug/g, poses a significant health threat to over 30% of the inhabitants to vitamin A deficiency. Enhancement of pro vitamin A/beta-carotene content in cassava is the most effective intervention strategy for addressing the vitamin A deficiency in sub-Saharan Africa.

Carotenoids are terpenoid compounds, a class of fat soluble pigments found primarily in plants, algae. Carotenoid compounds comprise lycopene, beta-carotene, zeaxanthin, and zeaxanthin-beta-glucosides. Genes such as phytoene synthase (PSY1), β-carotene Hydroxylase (HYD1), Lycopene β and ε cyclase (LYCB and LYCE), have been found to play a role in increasing levels of beta- carotene in plants. The genes and their products are useful for the conversion of farnesyl pyrophosphate to carotenoids. Marker-assisted breeding can be deployed in enhancing the nutritional value of many important staple foods such as cassava. The identification of genes and alleles that modify the beta-carotene is the most direct approach using genomics for plant breeding.

When we started this project, there was no cassava genome sequence. Therefore, we devised a comparative genomics approach to sequence and characterize partial sequence of genes implicated in pro-vitamin A biosynthesis pathway in cassava. In this approach, carotenoid sequences from the castor bean genome were used to conduct a BLASTn algorithm in a local cassava EST database which afterwards served as first set of primers. And second set of primers were designed from pVAC sequences obtained in Phytozome Manihot esculenta 5.0, as a result of a BLASTn algorithm with putative carotenoid ESTs. These primers were used to genotype several cassava genotypes of varying carotenoid content. Therefore our work focus on identifying single nucleotide polymorphism (SNP) attributed to

variation in carotenoid concentration among various cassava genotypes.

Materials and Methods

Plant material and evaluation of carotene content

Plant materials were selected from the cassava breeding program of International Institute of Tropical Agriculture (IITA) which developed advanced clones derived from a cross between yellow root and white root cassava. The total carotene content for these clones at the clonal evaluation stage, were evaluated using the standard Harvest Plus procedure. At the uniform yield trial stage (UYT), the quantitative levels of ß-carotene content in samples of fresh roots of the cassava clones were analyzed by COVANCE Lab, USA.

Selected plant materials and Genomic DNA Extraction

Initial trials were carried out on 32 lines with the first set of primers from ESTs and a panel of 40 clones set up afterwards with the 32 lines inclusive. All clones were collected in the uniform yield trial (UYT) stage and selected base on their total carotene content to constitute a panel of high carotene (6 to 15µg), low carotene (0 to 5µg) and white checks. Genomic DNA was extracted from 0.2g of fresh leaf of the selected plant materials using a modified Dellaporta method (Dellaporta *et al*., 1983). Quantification was done with spectrometer ND-1000, and then fractionated on 1% (w/v) agarose gels.

Target Genes, Primer Design and Initial PCR Amplification of Putative Carotenoid Genes.

Genes involved in the study and their abbreviation are: b- carotene hydroxylase(HYD1), Phytoene synthase (PSY1), lycopene epsilon cyclase (LYCE), and Lycopene beta cyclase (LYCB). In the initial experiment, Primers were designed from cassava ESTs (accessions; LYCE DB947186, PSY1 BH794911.1, LYCB DV445903, HYD1 DB955222), using BLAST algorithm (Altschul *et al*., 1997) performed with putative carotenoid genes from Castor Genome Database (http://www.tigr.org/msc), given the homology of castor bean and cassava. The second set of primers were designed from carotenoid gene (accessions: cassava7257.valid.m1, cassava992.valid.m1, cassava32745.m1, and cassava43823.valid.m1) of the cassava genome database (Phytozome v5.0 Manihot esculenta) (www.phytozome.net/cassava), for HYD1, LYCB, PSY1, and LYCE respectively) using BLASTx algorithm performed with putative carotenoid sequences of cassava obtained with initial primers.

PCR Amplification

The PCR was performed in volumes of 25µl reaction with 200ng/µl DNA template, 10x buffer, 50mM $MgCl_2$, and 2.5µM dNTPs, 1 unit of Taq polymerase, 4-5% DMSO, and 5% pmole of forward and reverse primers. Amplification was carried out with a touchdown program performed on a BIORAD peltier Thermal cycler with initial DNA denaturation at 94C for 2 min, followed by 9 cycles of 93C for 15 s, at -1C per cycle, and 72C for 30 s followed by 24 cycles of 93C for 15 s, 55-65C for 20 s, and 72C for 30 s. A final extension step was performed at 72C for 5min. PCR products were fractionated on 1.5% agarose. The primers were tested to genotype three accessions and primers that amplified were used for further trials.

Sequencing and Sequence Analysis

Amplified products with single bands were purified and directly sequenced using the two primers originally developed to amplify them. Sequencing was performed on ABI sequence analyzer (Applied Biosystems) and transferred to CodonCode Aligner (v2.0.6) for sequence editing and SNP analysis, Multiple alignments were performed in BioEdit version 7.0.0. Sequences were examined for polymorphism, as indicated by superimposed peaks in a chromatogram. Sequences were compared with other homologs in NCBI database using Blastn options (http://www.ncbi.nlm.nih.gov/blast). The sequences that had significant matches were considered as putative genes

Genotyping Population at Each Putative Carotenoid Biosynthetic Gene

The panel of 32 cassava lines obtained from the Uniform Yield Trial stage (UYT) of the cassava breeding program of IITA, with varying carotenoid [high (6-15µg), low (0-5µg)] content and white root advanced clones were genotyped using gene specific primers designed from cassava ESTs and directly sequenced. Single nucleotide polymorphisms (SNPs) were validated and used for further analysis. Heterozygotes were evident by the presence of a double peak at the SNP site in the sequence chromatogram.

Results and Discussion

Primer Design and PCR Amplification

Carotenoid genes from the castor bean genome database (http://www.tigr.org/msc) were compared with local cassava EST database using the BLASTn algorithm. The first set of primers was designed from sequences generated. The candidate EST sequences were used as query sequences in the cassava genome database (Phytozome Manihot esculenta 5.0), and the matching sequences (mRNA transcripts) were used to design several cassava specific primers for each candidate gene. The primers were designed at different points on the mRNA transcript to ensure a greater probability of amplifying a SNP at that

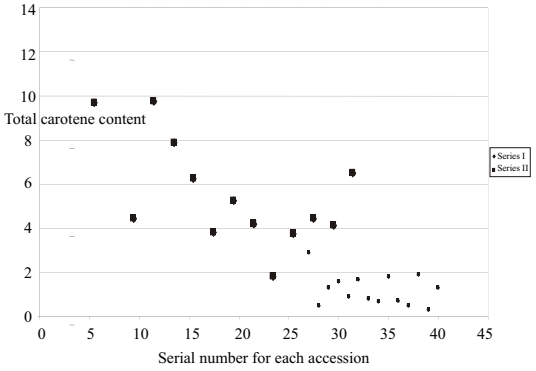

Figure 1. The numbers of clones used are represented here together with their total carotene content. Clones with carotene concentration of 6-15ug are regarded as high carotene lines

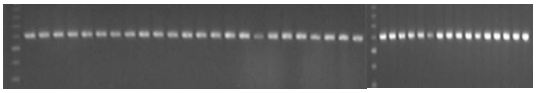

Figure 2. PCR Amplification with LYCE primers, run on 1.5% agarose gel gave a fragment size of about 550bp

Sequencing and Sequence analysis

Searches of sequences with homologs in other species on NCBI Basic Local Alignment Search Tool (BLAST) database (http://www.ncbi.nlm.nih.gov/blast/) using Blastn options revealed matches with 82%, 88%, 73%, and 92% similarity to sequences of HYD1, PSY1, LYCE, and LYCB, respectively (Table1). Bands of the appropriate size were purified and sequenced, and analyzed on Codon Code Aligner (v2.0.6) for identification of nucleotide variations among the sequences. A total of 228 sequences were generated across all genes (HYD1, 49; LYCB, 62; PSY1, 59; LYCE, 58). Polymorphic sites were identified (2 for LYCB, 15 for LYCE, 10 for HYD1 and 5 for PSY1), which were then called for the presence of usable SNPs (Table 2)

These sequences were considered to be putative cassava carotenoid genes. The high percentage of similarity of the matches indicates that these genes actually represent the enzymes in the carotenoid pathway responsible for accumulation of beta-carotene found in cassava. A summary of the first two closest putative ortholog and their percentage identity is given in Table 3.

Table 1. Percentage of NCBI accessions matching our putative caroteniod gene sequences using BLAST in algonithm in NCBI

Target Genes	Total # of Blast Hits	# Hitting target	# not Hitting target	% Blast Hits
HYD1	152	125	27	82
PSY1	125	111	14	88
LYCE	177	130	47	73
LYCB	100	92	8	92

Table 2. List of polymorphic sites and SNPs showing nucleotide variation identified in genes LYCB, LYCE, HYD1, PSY1

Gene	Total positions	Putative positions	# of Heterozygous positions	Max # of bases
LycB	2	0	1	2
LycE	15	7	0	3 (T,C,G)
Hyd 1	10	7	7	2
PSY1	5	1	0	2

Table3. BLASTn results from NCBI database showing accessions of closest putative ortholog producing significant alignment with putative carotenoid sequences of cassava

Enzyme name/ Gene symbol	NCBI Accession	Description	Query coverage	E-value	Identity
Beta-carotene hydroxylase	GU120076.1	*Manihot esculenta* beta-carotene hydroxylase (bHyd) mRNA, partial	37%	8e-63	100% (138/138) (49/54)
(HYDB1)	EF568375.1	*Manihot esculenta* beta-carotene hydroxylase 2 (bHyd) mRNA, partial	38%	4e-41	89% (121/136) (58/65)
Lycopene beta cyclase	GU120074.1	*Manihot esculenta* lycopene beta-cyclase (Lycb) mRNA, partial cds	100%	0.0	99% (465/469)
(LYCB)	EF568376.1	*Manihot esculenta* lycopene beta-cyclase (lycb) mRNA, partial cds	100%	0.0	98% (464/469)
Phytoene synthase	XM_002527022.1	*Ricinus communis* Phytoene synthase, chloroplast precursor, putative, mRNA	69%	2e-53	84% (181/221), (157/193), (65/77)
(PSY1)	GU111715.1	*Manihot esculenta* cultivar CM3306-4 phytoene synthase 2 (PSY2) gene, PSY2-W-1 allele, complete cds	32%	1e-24	72% (166/229)
Lycopene epsilon cyclase	XM_002514090.1	*Ricinus communis* Lycopene epsilon cyclase, chloroplast precursor, putative, mRNA	66%	4e-48	86% (220/294) Gaps = 24/294 (37/43) Gaps = 0/43
(LYCE)	AF117257.1	*Arabidopsis thaliana* lycopene epsilon cyclase gene, complete cds	69%	3e¯48	74% (251/337)

Sequence comparison with established conserved domains

Conserved motifs of carotenoid genes were identified using the Conserved Domain Database of NCBI (www.ncbi.nlm.nih.gov/cdd) and these motifs were identified in our putative cassava carotenoid sequences, indicating that the pathway can be characterized in cassava. Comparison of these sequences after multiple sequence alignment revealed regions of conserved histidine cluster motifs (Fig. 3) that contain histidine residues: HXXX(X)H, HXX(X)HH, and HXXHH, characteristic domain of the â-carotene hydroxylase superfamily.

Figure 3: Representative alignment of sequences showing the histidine cluster motifs that contain histidine residues: HXXX(X)H, HXX(X)HH, and HXXHH, characteristic domain of the β-caratine hydroxylase superfamily. Only the most conserved regions of the alignments are shown.

Multiple Sequence Alignment of Amino Acids

The nucleotide sequences of the putative carotenoid genes were subjected to multiple sequence alignment and nucleotide sequences were translated to amino acid sequences in selected frame using BioEdit software to identify variations within the amino acid sequences. A variation was found in the amino acid sequences of HYD1, but it occurred in both high and low carotene lines. Those found in PSY1 occurred in white, low and high carotene lines, but one occurred in only white line 30572; also, those found in LYCB occurred in white, low and high carotene lines. In LYCE lines, variations were found that occurred only in high carotene lines at different positions in the amino acid sequence in two cassava accessions namely 07/0539 and 07/0481 (Table 4). The variation found in the high carotene lines of LYCE is noteworthy since it was related to increased carotene content trait.

Multiple sequence alignment of LYCE sequences revealed the identification of a variation at position $G_{829}T$ in the nucleotide sequences in the high carotenoid accession (07/0539), and when aligned further with the reference transcript sequence for LYCE (cassava43823.valid.m1, Phytozome Manihot esculenta V5.0). The nucleotides were translated to amino acid to ascertain if the variation is reflected in the amino acid, and was identified at position $G_{349}C$ (Fig. 4). This genetic variation in the accession line with high carotene content is considered important, implicating its potential role in the accumulation of beta-carotene. Different source germplasms to together with linkage mapping will help to ascertain this haplotype.

Table 4. Summary of SNP variations in pVAC amino acid sequences

Gene	Primer	SNP	Position	summary
HYD1	1HYD1	F	159	Found in high lines and low 07/0520
PSY1	1aPSY	K	153	Found in white, low and high lines
		L	161	Found in white, low and high lines
	2PSY1	L	163	White line only TME1
		S	170	White line only TME1
LYCB	2LYCB	Q	16	Found only in the white line 30572
		L	70	White, low and high lines
		E	105	White, low and high lines
LYCE	2LYCE (R)	C	179	Only in a high line 07/0539
	3LYCE (F)			
		F	162	High line only 07/0481

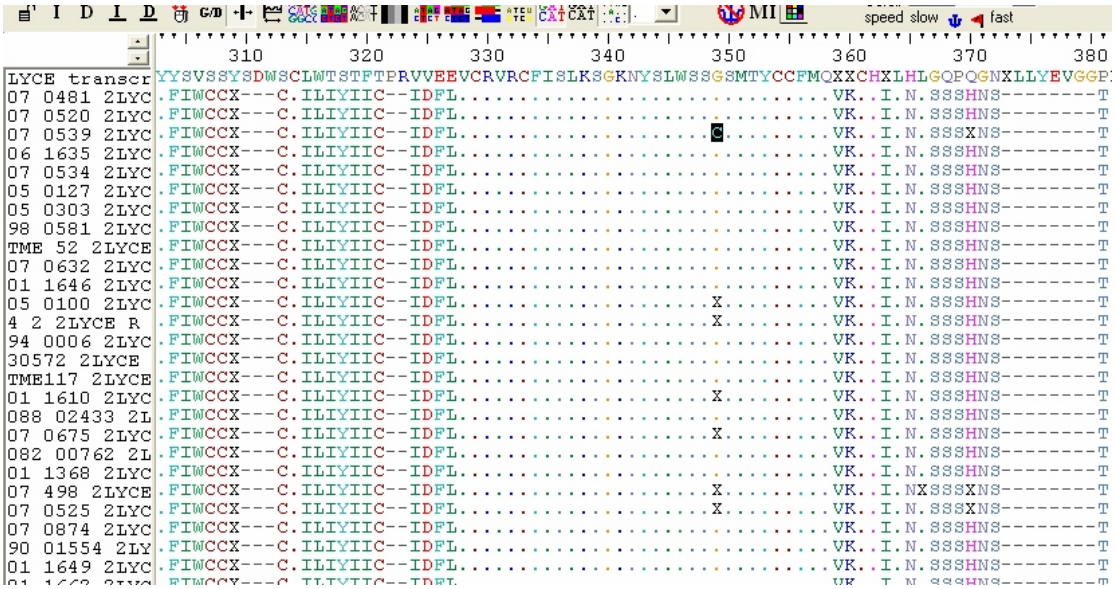

Figure 4. Amino acid alignment of LYCE sequences consisting of high and low carotene lines, including white lines with transcript sequence from Phytozome *Manihot esculenta* V.5.0, showing an amino acid variation in the high carotene line 07/0539. The relevant G349C amino acid exchange is shaded dark.

LYCE have been reported to be of critical value in the carotenoid pathway for β-carotene accumulation. A HPLC analysis showed lycopene to be the major carotenoid in yellow rooted cassava although ά-carotene and cis-lycopene were also found (Nassar, 2009).

Recently, through association analysis, linkage mapping, expression profiling and mutagenesis, it was demonstrated that variation at lycopene epsilon cyclase gene (LYCE) favorably alters the flux down alpha and beta-carotene branches of the pathway (Harjes *et al*., 2008). Most recently, to understand the biosynthetic pathway in cassava, Welsch *et al*., (2010) undertook comparative research using carotenoid-accumulating and white-rooted cultivars and obtained an allelic variation in phytoene synthase 2 (PSY2, another enzyme in the carotenoid pathway) of the yellow-rooted cultivar, which was found to cause a non-conservative amino acid exchange that led to markedly increased carotenoid formation and accumulation in cassava storage roots after transformation.

This approach, when completed, will contribute to enhanced genetic improvement of nutritional quality of cassava roots and an insight into the genetic and molecular basis of carotenoid biosynthesis. Discovered genetic variants will be validated and ultimately converted into user friendly assay for marker-assisted breeding. The partial sequences available also serves as a powerful tool for future study of carotenoid biosynthesis in cassava and other species and can be useful in devising transgenic approaches to manipulate carotenoid content in cassava and related plants (Fraser *et al*., 1999; Rosati *et al*., 2002). In this regard, the development of molecular marker to easily identify carotenoid accumulating genotypes will be important since it shortens the work of the breeder in carrying out recurrent selection.

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Variation in micronutrient content of orange-fleshed sweetpotato (*Ipomoea batatas* (L.) *Lam.*) varieties grown in different environments of Region III of Zambia

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Abstract

Sweetpotato is one of the most important sources of carbohydrates among small-scale farmers in Zambia and ranking second only to cassava. A study was conducted under field conditions at three locations during the 2008/09 season to determine the variability of micronutrients and to characterise the agronomic traits of orange-fleshed sweetpotato varieties grown under different environments. The experiments were laid out and evaluated in a RCBD with 3 replications. A total of 15 varieties, including 2 local varieties, were used. Analysis of variance results showed that there were significant differences in the locations for zinc with highest content obtained at Kamato with 44 mg/100g followed by Mansa with 27 mg/100g and lastly 12.8 mg/100g at Mutanda, while varieties were significantly different (P=0.05) for iron. There was differential response of the varieties to the locations with regards to iron. Naspot1 and Ukerewe had the highest iron concentration at 11.20 mg/100g and 8.06 mg/100g. It was also revealed that locations, varieties and interactions were significantly different (P?0.05) for â-carotene and vitamin A concentrations of sweetpotato. The variety Zambezi, K566632, 199062.1 and Mayai produced high mean concentration of â-carotene. These were 7.82 mg/100g, 7.89 mg/100g, 6.18 mg/100g and 6.52 mg/100g, respectively. For marketable yield, locations and varieties were significantly different at P=0.05. The varieties with the highest marketable yield were Naspot1 and K118 at 13.74 t/ha and 9.57 t/ha, respectively. The variety with the lowest marketable yield was Kakamega at 5.23 t/ha. The mean marketable yield for locations ranged from 5.23 t/ha at Mansa to 9.69 t/ha at Mutanda. The results for non-marketable yield showed that locations, varieties and interactions were significantly different at P=0.05.The highest non-marketable yield varieties were Kalungwishi and 199062.1 with 7.26 t/ha and 5.50 t/ha respectively. There was a differential response in non-marketable yield for varieties tested as evidenced by interactions. None of the high yielding varieties showed high levels of stability. Varieties were also significantly different for harvest index. The varieties with high harvest index were 199062.1, Carrot.C and Mayai at 85%, 83% and 83% respectively. Gweri, Kakamega and Pipi had the lowest HI with values of 67%, 68% and 70%, respectively.

Keywords: Sweetpotato, variability, environment

Introduction

In Zambia, sweetpotato is the second most important root crop from cassava and has the potential to contribute significantly to food security as a source of energy and vitamin A (Chiona *etal*, 2007). Micronutrient due to micronutrient deficiencies, such as Fe and Zn, causes blindness and anemia in more than half of the world population, especially in women and pre-school children of South and Southeast Asia and Sub-Saharan Africa (Reddy *et al.,* 2005). Attempts have been made to alleviate these deficiencies by the use of supplements and food fortification, but these strategies do not reach all those suffering from deficiency and have not proven to be sustainable (Römheld, 1998). There are a number of reasons sweetpotatoes biofortified with iron and zinc could be a powerful tool in the fight against Fe and Zn malnutrition. Sweetpotato is an important staple crop in areas in which iron and zinc deficiencies were a particular problem (Courtney, 2006). Sweetpotatoes provide a large yield per area per unit of time, and are capable of yielding even in marginal conditions. This makes it an ideal sustainable crop for production in developing countries, where high population has decreased growth the amount of arable land per person and results the use of marginal land for food production (Woofe, 1992). Presently little is known about the concentration of iron and zinc in sweetpotato. A range of 0.59 mg/100g to 0.86 mg/100g (fresh weight) and a level of 0.24 mg/100g (fresh weight) for Fe and Zn, respectively, were given in Woofe (1992).

Orange fleshed sweetpotato varieties are rich in beta carotene that the body uses to produce vitamin A Vitamin A deficiency weakens the immune system leaving them susceptible to diseases such as measles malaria and diarrhea

Hegenimana et al To generate sufficient supply of micronutrients through diets mainly consisting of sweetpotato specific interventions in plant breeding are needed

The objectives of this study were to characterise the agronomic parameters and to determine if there are genetic variations in micronutrient concentrations of orange fleshed sweetpotato varieties grown under different environments

Materials and Methods

The experiment was conducted in three different locations of Region III in Zambia for one planting season This was mainly due to limited availability of planting material The experiment was planted at Mansa Research Station on th January Mutanda Research station on st January and Kamato Solwezi on st February Mansa Research Station is located Km east of Mansa Town Mutanda Research Station and Kamato are located km west of Solwezi town Each site differs for climatic characteristics Genotypes were evaluated in a Randomized complete block design with three replicates in a plot size of m at spacing of cm within the row and m between rows

The experiment consisted of orange fleshed sweetpotato varieties The criteria used to choose the varieties was mainly based on availability of planting material and also this is in line with the partnership forged between International potato center CIP and the Zambian government through the VITAA Vitamin A for Africa initiative to disseminate and promote production of orange fleshed sweetpotato OFSP varieties Planting was rain fed and carried out for each location when there was sufficient moisture to sustain good plant establishment Healthy vines cm long were planted on ridges in a slanting position with two thirds of the vine length buried in the soil The fields were maintained free of weeds and no herbicides were applied A basal dose of Kg N Kg P O and Kg K O per ha was applied using compound D Additional Kg N per ha was top dressed when the plant was weeks old The tubers were harvested at months after planting in each site and yield was based on the net plot

Data collected included agro ecological (site) description data, monitoring data and data at harvest. The trials were harvested 4 months after planting. Yield of marketable and non-marketable tubers / plot were also determined at harvest. Harvested roots were washed in tap water and allowed to air-dry before weighing. They were then rinsed in tap water, peeled with a knife, and rinsed in tap water again. Dry matter content (DM) was determined after weighing 200grams and oven- drying to a constant weight at 70°C. DM of storage roots was expressed as the average percentage of dry weight of fresh weight. Skin and flesh colour was recorded using the standard sweetpotato colour sweetpotato chart (Kapinga *et al.,* 2010).

The harvest index was calculated at harvest as a ration of tuber yield to the total biological yield and expressed as a percentage. Only the below-ground portion of the crop was used in the calculation. Soil and variety samples were collected from three locations and analysed for zinc and iron content.

Statistical analysis

Analysis of covariance (ANCOVA) was carried on plot mean basis using procedures of the GenStat statistical package developed by VSN International Ltd. Further characterization of the G X E interaction for yield was done using AMMI model (Gauch, 1992). The stable varieties identified according to the interpretation given by Crossa *et al*. (1991) that ordinates for two principal components plotted against each other, entries near the centre are average in the performance.

Results and discussion

Analysis of covariance (ANCOVA) (table 1) indicated significant (P= 0.05) mean squares for storage yield, non-marketable yield, Harvest, Index, beta-carotene, vitamin A and Iron content for all sources of variation. Marketable yield was significant for genotype and not significant for GxE. Dry matter content and Zinc content were not significant for GxE and for genotype.

Table 1: ANCOVA table for combined mean squares for all variables measured across three sites

Source of Variation	DF	Yield (t/ha)	Mrkt.yield. (t/ha)	Non-Mrkt.yield. (t/ha)	HI (%)	DM (%)	β-Car. mg/100g	Vit .A ug RE/100g FW	Zinc mg/100g FW	Iron mg/100g
Rep.										
Covariate	1	24.0	20.9	7.8	0.039	1.8	2.8	18325	1.11	3.6
Residual	1	3.0	36.2	1.1	0.001	19.8	0.2	1558	0.34	0.4
Environ.	2	78.7*	19.9*	26.7*	0.182*	8.8ns	189.6*	1316910*	103.0*	2.9ns
Genotype	14	87.6*	37.6*	19.9*	0.022*	19.8ns	31.4*	217533*	1.3ns	8.9*
G x E	28	25.5*	19.5ns	10.4*	0.033*	11.4ns	10.2*	70684*	2.0ns	6.0*
Covariate	1	3.2	57.8	17.2	0.145	4.1	2.8	19292	1.2	1.0
Residual	87	15.3	11.9	3.6	0.145	11.8	2.1	14606	1.2	2.1
CV %		31.7	42.7	29.7	13.1	12.6	53.7	53.7	25.2	27.2

*significant at Pd0.05

NS Non significant

The varietal differences observed above reflect the wide spectrum of the root flesh color of sweetpotato. Woofe (1992) and Low *et al*. (1997) suggested that cultivars having more than 100 ug/100g (1.0mg/100g) retinol were good sources of vitamin A. The picture with regards to Vitamin A is similar to that of beta-carotene on the basis that the predominant carotenoid in sweetpotato roots is beta-carotene which represents the main source of provitamin A in the roots (Takahata, 1995).

Table 2 shows that the Zn contents of soils from Mutanda, Kamato and Mansa were considerably higher than the critical value of 0.8 ppm indicated by Lindsay and Norwell (1969). It also shows that Zn levels in sweetpotato are not dependent on the genotype. The results obtained in this study are similar to the findings reported by Courtney, (1996) who reported a range of 1.58 mg/100g to 3.67 mg/100g.

Courtney (1996), found clear trends of Zn in fresh sweetpotato to vary significantly among genotypes, which is contrary to the current study results where no differences among varieties were detected. The failure to detect differences among entries in the current study can be linked to the use of limited sample of sweetpotato genotypes and few locations within a single season.

Courtney (1996), found Fe in fresh sweetpotato to vary significantly among genotypes, which is similar to the current study results where significant differences among varieties were detected.

The results showed variations in the concentration of both elements in the varieties, with varieties exhibiting higher Zn concentration than the soils. The Fe content of sweetpotato ranged from 2.9 mg/100g to 11.20 mg/100g. Table 2 shows that the Fe contents of soils from Mutanda and Kamato were considerably higher than the critical value of 4.5 mg/100g indicated by Lindsay and Norwell (1969) whereas the Fe contents of soil from Mansa was considerably lower than the critical value. The current study shows similar results from (Reddy *et al.* 2005**)** who found concentration of Fe in the ash of needles and twigs, with each exhibiting lower concentration than the soils. The dry matter levels obtained in the current study were similar to those reported elsewhere (Courtney, 1996) who reported a range of 21.33-42.2 %. Kapinga *et.al.* (2005), on the other hand, found dry matter of orange fleshed sweetpotato to vary among varieties contrary to the current study results and the levels were in the range 34.9% - 36.5%. The failure to detect difference among entries for DM in the current study could be due to the homogeneity, with respect to of the genotypes tested.

Table 2: Geographic and soil physic-chemical characteristics of the different sites used in the study.

District	Location	Rainfall y	Altitude y	Soil texture	pH	N	Fe (mg/100g) x	Zn (mg/100g) x	Cu (mg/100g) x
Solwezi	Mutanda	950 mm	1400 m	Sandy loam	5.5	0.145	90	<1	4
Solwezi	Kamato	950 mm	1400 m	Sandy clay loam	4.5	0.145	331	1	8
Mansa	Mansa	-	1400 m	Sandy loam	6.0	5.7	0.870	4.73	0.78
Key for interpretation									
High					5.5 -6.5	>0.30	4.5	0.8	>2.0
Low					<4.5 -5.0	0.10	<4.5	<0.8	2.0

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[y] Metrological Department of Zambia

Conclusion

From the current study it can be concluded that selection for high yielding orange-fleshed sweetpotato varieties for the varying environments is feasible though identification of superior varieties in terms of zinc and iron will require use of large samples of materials tested over a number of seasons and preferably over a number of locations.

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Participatory breeding for Pro-Vitamin A - enriched cassava in Nigeria

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Abstract

The enrichment of cassava with pro-vitamin A through breeding and selection, also known as biofortification, has resulted in several new varieties of cassava with enhanced levels of the micronutrient. Breeding for enhanced pro-vitamin A should ensure that the traits that enhance high productivity and strong end-user preferences. Twenty two high pro-vitamin A cassava (pVAC) or yellow root varieties in advanced trials and two elite varieties which have white roots were used to determine their productivity and to assess end-users' preferences for them through participatory approaches. The fresh root yield ranged from 15 to 47 t/ha, dry root yield from 3.7 to 14 t/ha, dry matter content from 13.5 % to 40.4 % and total carotene from 0.96 to 8.13 µg/g, indicating wide variability suitable for further trait improvement. Ten farmers were used to evaluate the perceptions and preferences of the varieties based on agronomic attributes and sensory evaluation through panel assessment. The parameters that influenced high preference for varieties like 05/1652, 05/0303 and 05/1601 and the check NR 8082 were plant type, size of roots, colour of fresh root pulp and bulking pattern of the roots. The parameters that influenced high preference for varieties such as 05/1631, 05/0127, 05/0473, 05/1646, 05/0327 and the check NR 8082 were colour of the dry and wet steeped gari, aroma, taste and texture of the steeped gari. Putting these into consideration in the breeding of high pVAC varieties would guarantee widespread farmer acceptance and maximum impact on nutritional health.

Keywords: Cassava, biofortification, pro-vitamin A, sensory analysis, farmers, participatory breeding

Introduction

Cassava is one of the crops targeted for biofortification as it is consumed daily by populations in Sub-Sahara Africa (Thakkar et al., 2009) and it has been estimated that 70 million people in Africa consume more than 500 kcal/day from cassava (Aerni, 2006). Consumers and farmers often prefer cultivars of good quality to a higher yielding cultivar of poorer quality. Some improved cultivars have failed in the market because they could not meet other requirements of the consumers (Mahungu, 1983). Wheatley and Gomez (1985) have drawn attention to the great variability among cassava cultivars in root quality factors (such as cooking time, taste, texture, hardness, starch content), and their dependence on plant age at harvest. Although its popularity as a staple compares with cereal grains in Northern climates, most cassava varieties are low in protein less than one percent, compared with about seven percent in commonly grown staple grains. Different cultivars of cassava have different food quality characteristics. Commercial cassava varieties are very low in essential micronutrients and minerals such as beta carotene, the precursor for vitamin A. There is need to develop varieties that will provide the minimum daily requirements for pro-vitamin A. Developing micronutrient-enriched staple plant foods such as cassava, either through traditional plant breeding methods or other modern techniques, is a powerful intervention tool that targets the most vulnerable people (resource-poor women, infants, and children (Bouis, 2000). Root quality traits usually considered in variety development include fresh yield, dry matter content, starch quality and cyanide potential (Uzokwe, 1998). Cassava could serve as a vehicle for alleviating vitamin A deficiency. In Nigeria, 70% of cassava consumed is in the form of gari (Ekwe, 2004). Such vitamin A enriched cassava will be useful in the gari consumed by at least 50 million Nigerians.

Conventional breeding as applied in many organizations are purely researcher oriented without reference to the end-users. The drawback in such crop improvement efforts is the exclusion of consumers and farmers in the evaluation process for new varieties. Participatory breeding is useful when producers and other stakeholders in a value chain significantly influence decisions on crop improvement and the kinds or quality of improved crops that are introduced into the food or agricultural system (Ashby, 2009). The resultant effect of this approach is that plant varieties that are well tailored to poor farmers' needs are developed in s short time and thus accelerating adoption and seed dissemination. The aim of this work was therefore to evaluate 24 cassava

varieties for tuberous root yield and determine their total carotene content in order to develop high yielding cassava varieties with high pro-vitamin A content in a participatory approach. This study will help in answering the question of acceptability of the yellow root colour of high pro-vitamin A cassava (pVAC) varieties in Nigeria.

Materials and Methods

This experiment was conducted at NRCRI, Umudike to evaluate 22 pVAC varieties for tuberous root yield and beta carotene content as well as farmers' and consumers' preferences for newly developed pVAC. Two white root genotypes, NR 8082 and 91/02324 were used as checks. The experimental design was RCBD replicated three times with each variety planted in a 6 x 6 m plot at a spacing of 1 x 1m giving a population density of 10,000 per ha. Data were taken on fresh root yield, dry matter content, and total carotene content among other parameters. Carotene content of the cassava varieties were determined using the Harvest Plus procedure (Rodriguez-Amaya and Kimura, 2004) for carotene determination in cassava. Three plants were harvested in each plot representing a variety on the field. Six root tubers (2 big, 2 medium and 2 small) were selected for each variety from among all the tubers uprooted in the plot and transported to the laboratory in big envelopes used to collect the samples immediately. The roots were pulverized and carotene extraction was done with acetone and petroleum ether. The absorbance for each sample was read at 450 nm. The total carotenoid content was calculated using the following formula:

$$\textit{Total caroteme content} = \frac{\text{A x volume (ml) x 10000}}{\text{A1\%1cm x sample wwight (g)}}$$

where A = Absorbance; volume = total volume of extract (25 ml); $A^{1\%}_{1cm}$ = absorption coefficient of carotene in petroleum ether (2592).

A group of ten cassava farmers were purposefully sampled from 3 major cassava growing communities in Abia State on the basis of earlier involvement in farm level research and extension activities of the Institute. Prior to the workshop day, 10 kg roots of pVAC cultivars were harvested and first processed into gari under the farmers' condition. Again, the toasted gari was further prepared into a readily consumable form by steeping a measure in hot water to form a paste. Both the toasted gari and the steeped gari made from the cultivars were evaluated by the farmers using 7 sensory attributes such as color of dry (pre-cooked) gari, colour of steeped gari, smell, taste, texture, fibre content, starchiness. During the evaluation farmers were encouraged to observe, touch, smell and taste the samples to ensure objective assessment. The assessments were made on a 4 maximum unstructured scale where 1 represented the "least preferred" and 4 "the most preferred" (Tomini et al, 2004). Panel assessment sessions were duly observed both for the field and sensory assessment of the pVAC cultivars.

The colour preferences of respondents revealed that light yellow *gari* product is more preferred to deep yellow and white ones, an rather subjective bias for the communities around the study area. It was also evident that colour attribute captured the interest and delight of the farmers which suggests high adoption potential for the pVAC cultivars that might be released eventually.

All data was subjected to analysis of variance using the SAS 9.2 version (2008) software while cluster and descriptive analyses were carried out on data collected using SPSS version 10.

Results and Discussion

Agronomic performance: The carotene content, dry matter content, fresh root yield and dry root yield of 21 yellow rooted cassava varieties and two checks (NR 8082 and TMS 91/02324) are shown in Table 1. The two checks are popular white root cassava varieties. The result showed that variety TMS 05/1632 had the highest carotene content of 8.13 µg/g while NR 8082 and TMS 91/02324 (the white checks) had the least values of 0.96 µg/g and 0.99 µg/g, respectively. There was high variation among the varieties for total carotene content, hence, the opportunity for further improvement of the trait in cassava. Variety TMS 05/0303 had the highest fresh and dry root yield of 64 and 20.44 t/ha, respectively (Table 1). Dry matter content of roots ranged from 13 to 40% with a mean of 26%, a reasonably broad range for pVAC varieties, and comparable to current commercial cultivars. Very high variability was observed among the varieties for all the variables evaluated.

Farmers' assessment of agronomic characteristics: An analysis of the panelists' preference ratings shows classification of the cultivars into three clusters on the basis of their preferences scores (Table 2). Their assessment for agronomic characters was based on the dominant attributes such as colour of fresh root pulp and plant type

with size, structure and bulking pattern of the roots were inclusive. Cluster 1 comprised 4 cultivars that were most preferred by the respondents as indicated in the respective means of their preference scores as follows: 05/1652(3.8), 05/0303 (3.7), 05/1601 (3.7) and the control cultivar NR 8082 (3.8). Cluster 2 is made of 16 cultivars that were moderately preferred by the respondents as indicated by the means of their preference scores. These include 05/1658 (3.3), 05/0100 (2.9), 05/1646 (3.4), 05/0127 (3.0), 05/0251 (3.3), 05/1625 (3.3), 05/1626 (3.3), 05/0473 (2.8), 05/1654 (3.2), 05/1631 (3.3), 05/1600 (3.4), 05/1599 (3.2), 91/02324 (3.0), 05/0327 (3.0), 05/1662 (2.9), and 05/1653 (3.0). Cluster 3 comprised 3 cultivars that were least preferred on agronomic characters by the respondents as shown by the means of their preference scores. Such cultivars include 05/1632 (2.4), 05/0186 (2.0) and 05/1636 (2.1). From the results, it was evident that the clusters differed significantly ($P < 0.05$) with respect to all agronomic attributes but there was no significant difference in mean scores of attributes among cultivars in each cluster.

Sensory analysis of gari from pro-vitamin A cassava cultivars: The cultivars were also classified into three cluster groups according to the preference scores of sensory attributes of the cultivars being evaluated (Table 3). The sensory attributes evaluated were dry gari colour , wet steeped gari colour, fibre content, aroma, taste, texture, starchiness. Of these the most influential were colour of dry gari and colour of gari (wet steeped) were the most dominant followed by taste and texture. Cluster 1 is made up of 6 cultivars that were the most preferred by the panelists as shown in their respective means scores. Cultivars such as 05/0127 (3.8), 05/0473 (3.9), 05/1631(3.7), 05/0327 (3.8), NR8082 (3.8) and 05/1646 (3.7) were in this cluster. Cluster 2 consists of 12 cultivars that were moderately preferred as shown by their mean scores including the check NR 8082. These include 05/0303 (3.4), 05/0100 (3.3), 05/1599 (3.5), 05/1652 (3.4), 9102423 (3.5), 05/1662 (3.5), 05/1653 (3.4), 05/1600 (3.4), 05/1654 (3.4), 05/0186 (3.4), 05/1636 (3.4), 05/1625 (3.5). Cluster 3 was made up of gari products from 5 cultivars that were considered as least preferred by the panelists as shown by the respective mean scores. These include 05/1658 (2.6), 05/1626 (2.9), 05/1632 (2.5), 05/1661 (3.2), 05/0251 (3.2).

The results specifically indicated that certain cultivars such as 05/1652, 05/0303 and 05/1601 and the control (NR 8082) were preferred to others on the basis of their morphological attributes. However, in terms of the sensory attributes, only the control (NR 8082) maintained the 'most preferred' status. Few other cultivars were rather rated alongside NR 8082 as the 'most preferred.' These include pVAC varieties, 05/1631, 05/0127, 05/0473, 05/1646 and 05/0327. This implies that only NR 8082 was consistently high in the preference ranking of both morphological and sensory attributes, probably due to its familiarity and popularity among the communities from which the panelists were drawn. Judging by the high rank scores for productivity, farmers' impression of their agronomic performance and sensory parameters, 05/1652, 05/1631, and 05/0473 could be considered as suitable candidate varieties for delivery to farmers. These had fresh root yields above 30 t/ha, dry yields above 10 t/ha, dry matter contents above 30% and mean total carotene of at least 6 µg/g. They could be used simultaneously in breeding for higher carotene contents in adapted varieties. Aside from the vitamin enrichment of the cassava certain criteria such as crop productivity, stability of the carotene content across various environments, bioavailability of the pro-vitamin A and consumer acceptance of cooking quality of the products formed from the cassava by household members (Welch and Graham, 2004). Putting these into consideration in the breeding of high pVAC varieties would guarantee widespread farmer acceptance and maximum impact on nutritional health.

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Table 1: Fresh root yield, dry root yield, dry matter content and carotene content of 24 cassava varieties evaluated at Umudike

Genotype	Fresh root yield (t/ha)	Total carotene content (µg/gFW)	Dry matter (%)	Dry root yield (t/ha)
05/0100	28.06	4.36	31.58	8.86
05/0127	45.85	5.02	28.66	13.14
05/0186	22.86	4.74	31.95	7.3
05/0251	31.81	5.32	27.06	8.61
05/0303	64	4.68	31.93	20.44
05/0327	30.33	4.47	30.23	9.17
05/0473	30.05	5.53	29.83	8.96
05/1599	34.08	5.22	25.45	8.67
05/1600	47.08	4.32	13.55	6.38
05/1601	42.67	6.75	28.48	12.15
05/1625	27.16	6.17	13.61	3.7
05/1626	29.05	6.69	19.87	5.77
05/1631	32.86	5.69	40.43	13.28
05/1632	15.25	8.13	33.67	5.14
05/1636	19.56	7.80	24.05	4.7
05/1646	44.26	5.70	17.88	7.91
05/1652	31.65	5.82	31.07	9.83
05/1653	45.44	4.75	24.98	11.35
05/1654	39.94	7.09	35.33	14.11
05/1658	29.23	5.67	31.43	9.19
05/1662	30.03	7.01	18.78	5.64
91/02324	31.76	0.99	24.9	7.91
NR 8082	47.02	0.96	21.92	10.31
Mean	34.51	5.34	26.5	9.08
St dev	10.69	1.74	6.86	3.72
CV (%)	30.99	0.36	25.89	40.98
Maximum	47.08	8.13	40.43	14.11
Minimum	15.25	0.96	13.55	3.7

Table 2. Participatory evaluation of consumers' preference ranking of pro-vitamin A cassava cultivars on the basis of agro-morphological traits

	Cluster 1 (Most preferred)	Cluster 2 (Moderately preferred)	Cluster 3 (Least Preferred)
1	05/1652 (3.8)	05/1658 (3.3)	05/1632 (2.4)
2	05/0303 (3.7)	05/0100 (2.9)	05/0186 (2.0)
3	05/1601 (3.7)	05/1646 (3.4)	05/1636 (2.1)
4	NR 8082 (3.8)	05/0127 (3.0)	
5		05/0251 (3.0)	
6		05/1625 (3.3)	
7		05/1626 (3.3)	
8		05/0473 (2.8)	
9		05/1654 (3.2)	
10		05/1631 (3.3)	
11		05/1600 (3.4)	
12		05/1599 (3.2)	
13		91/02324 (3.0)	
14		05/0327 (3.0)	
15		05/1662 (2.9)	
16		05/1653 (3.0)	

Values in parenthesis are mean score of farmers' preference ranking. Ranking based on plant size, branching pattern, root size, root structure, root colour, root moisture content, bulking pattern.

Table 3. Participatory evaluation of consumers' sensory preference for pro-vitamin A cassava cultivars.

	Cluster 1 (most preferred)	Cluster 2 (moderately preferred)	Cluster 3 (Least preferred)
1	05/1631(3.7)	05/0303 (3.4)	05/1658 (2.6)
2	05/0127 (3.8)	05/0100 (3.3)	05/1626 (2.9)
3	05/0473 (3.8)	05/1599 (3.5)	05/1632 (2.5)
4	NR 8082 (3.8)	05/1652 (3.4)	05/1661 (3.2)
5	05/1646 (3.7)	9102324 (3.5)	05/0251 (3.2)
6	05/0327 (3.8)	05/1662 (3.5)	
7		05/1653 (3.4)	
8		05/1600 (3.4)	
9		05/1654 (3.4	
10		05/0186 (3.4)	
11		05/1636 (3.4)	
12		05/1625 (3.5)	

Values in parenthesis are mean score of farmers' preference ranking.Ranking based on dry gari colour, cooked gari colour, fibre content, aroma, taste, texture, stickiness.

Evaluation of G x E interaction of sweetpotato for beta-carotene content, root dry mass harvest index, vine fresh yield and root fresh yield.

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Abstract
The effect of genotype (G) by environment (E) interaction (G x E) on β-carotene content, root dry mass (RDM), harvest index (HI), vine fresh yield (VFY), and root fresh yield (RFY) of 15 selected progeny from a polycross were investigated at five diverse locations in Zambia. The locations represented the major sweetpotato growing agroecologies in the country. The objective was to identify stable and high performing genotypes. The G x E analysis was conducted with the additive main effects and multiplicative interaction model (AMMI). The performance of genotypes was dependant on location for all the traits considered.

The magnitude of the G x E for β-carotene content, RDM, and HI was small and selection for these traits may be conducted in a few, well selected environments. Conversely, RFY and VFY yield may require early testing in varied environments to select genotypes with either wide or specific adaptation.

The AMMI analysis identified progeny G2, G6, and G8 as stable with above average performance across environments for β-carotene content (5.0, 4.7, and 4.7 mg 100 g^{-1}, respectively), RDM (37, 37, and 35%, respectively), HI (0.7, 0.6, and 0.7, respectively), and RFY (14.2, 13.0, 14.4 t ha^{-1}, respectively). Genotype G3 was specifically adapted to environment E3, E4, and E5 for β-carotene content, RDM, and RFY. It had the highest mean â-carotene content (9.4 mg 100 g^{-1}), high mean RDM (35%), and high RFY (14.7 t ha^{-1}) across the environments. It was concluded that it is possible to breed for high β-carotene, high RDM and high yield sweetpotato genotypes with wide or specific adaptation in Zambia as the AMMI analysis identified genotypes G2, G6, and G8 as stable across environments for both β-carotene content and RDM. They performed above average for both traits.

Introduction

Genetic adaptation entails the shaping of a population or a species gene pool in response to environmental challenges (Perez-de-la-Vega and Tigerstedt, 1996). A crop's ability to exploit its environment depends on many adaptive features that are controlled by multiple genes, interacting among themselves and with the environment in intricate ways (Hawtin et al., 1997). The genotype by environment interaction (G x E) observed by plant breeders signifies differential responses of the cultivars being tested to different environmental conditions and is a major challenge in plant breeding (Ceccarelli and Hammer, 1996). In essence, G x E reduces the correlation between the phenotype and the genotype.

Ceccarelli et al. (1994) suggested that if the G x E is of the crossover type, genotypes developed in favorable environments do not perform well under harsh environments and vice versa. This suggests that the genes for yield expressed in low and high input conditions are different. As a result, breeding procedures conducted under high input and uniform agronomic conditions might favour selection of cultivars adapted to intensive management and might eliminate genotypes adapted to low input conditions (Ceccarelli, 1997). Crossover interaction causes problems in crop breeding because it hinders selection progress due to changing composition of genotypes selected in different environments (Cooper and Delacy, 1994; Crossa et al., 1995). Other workers (Braun et al. 1997; Eberhart and Russell, 1966; Troyer, 1996), however, have suggested that it is possible to breed for wide adaptation provided that the genetic base is broad enough.

It was against this background that this study was designed to determine the adaptability of sweetpotato genotype across locations for b-carotene content, root dry mass (RDM) composition, harvest index (HI), vine fresh yield (VFY), and root fresh yield (RFY) to determine the magnitude of the effect of G x E on these traits, and to identify stable and high performing genotypes.

Materials and Methods

Polycross mating design

Sweetpotato genotypes with high â-carotene content and high RDM were open-pollinated in two field grown polycrosses (Plate 1) established at Mansa Research Station (11° 14.4' S and 028° 57.2' E), Zambia in December 2005. The high β-carotene germplasm was introduced from the Vegetable and Ornamental Plant Institute, Roodeplaat, South Africa and from the International Potato Centre (CIP) in Kenya. The high dry mass germplasm was obtained from the sweetpotato breeding programme in Zambia (Appendix 1). The first of the two polycross had 12 parents planted in a randomised complete block design with 12 replications. The second polycross had 30 parents planted in a randomised complete block design with eight replications. Both polycrosses were planted in areas sufficiently isolated from other sweetpotato plants. Data on plant establishment, vigour, flowering, seed set, and number of seeds produced per parent was collected.

From May to July 2006, seed was collected from the parents. The seed was cleaned by hand and stored for two months in paper bags under room condition in readiness for germination. Prior to planting, the seed was first scarified by immersing in concentrated H_2SO_4 (98%) for 20 minutes (Rossel et al., 2008). The scarified seed was sown in wooden boxes filled with black top soil which were placed in a screen house. Once the seedlings had reached 50 mm in height, they were removed from the screen house and transplanted to the nearby wetland on 10 m long by 1 m wide ridges. The available water in the wetland enabled good seedling establishment. Macro nutrients ($10N\text{-}20P_5O_2\text{-}10K_2O$) at a rate of 100 kg ha^{-1} were added to the soil in the wetland to boost vegetative growth of the transplanted seedlings.

Progeny screening

In November 2006, the cuttings from the wetland were planted in the field for evaluation. Cuttings provided a source of potential cultivars and were screened in an observational single plant trial. Cuttings were planted in groups according to family. The plants were screened for pests and diseases and other defects. At maturity the surviving plants were evaluated for good root traits, namely: shape and size, root neck length and root flesh colour. Flesh colour determinations were made using the 1995 edition of the Royal Horticultural Society (RHS) Colour Chart (Royal Horticultural Society, 1995).

Progeny with desirable characters (orange-fleshed, high RDM, field resistance to major pests and diseases) were selected. The threshold values for selection were predetermined as follows:

1. medium to dark orange root flesh colour (RHS:9 137 U or better)
2. High RDM (above 30%)

3. Marketable root yield (above 120 g root^{-1})

Progeny that did not exceed the threshold in any one trait were discarded. In total, 1470 progeny were evaluated and 35 progeny met the selection criteria. The selected progeny were maintained in the wetland (Plate 2), and at the same time multiplied to increase the vines for planting.

Plate 1 Polycross conducted at Mansa Research Station, Zambia

Plate 2 Genotypes from seedlings growing in the wetland area

Field trial evaluation of selected progeny for G x E

In November 2007, replicated trials were established in two different locations, namely: Mansa-Mufulira (11° 06'S and 28° 51'E) and Mutanda West (12° 24'S and 26° 15'E) (Appendix 5), using 15 of the 35 selected progeny from the previous season (Table 1). The criterion on which the 15 progeny were selected was based on genotypes being able to provide at least 500 tip cuttings to ensure enough planting material for the two locations. The remaining progeny were multiplied and evaluated separately. The trial was repeated in 2008 at three locations, namely: Mansa-Main (11° 14.4' S and 028° 57.2' E), Mutanda East (12° 11'S and 26° 24'E), and at Golden Valley Research Trust (GART) (10° 07'S and 30° 55'E) (Appendix 2). A randomised complete block design with three replications was used for all the trials. The experimental plot comprised four 6 m long ridges spaced at 1 m. Plants were spaced at 30 cm within each ridge. The two middle ridges were used for data collection and plants on outer ridges were not used. During plant growth, observations were made for any pests and diseases and other biotic stresses. At harvest number of roots, and RFY and RDM composition were determined. Five plants from the central two rows of every experimental plot were randomly selected at harvest time to generate subsamples for root dry mass and β-carotene content determinations. The β–carotene content was determined by the South Africa Bureau of Standards, in Pretoria, South Africa in 2008 and by the Tanzania Food Nutrition Center, Dar-es-Salam, Tanzania in 2009 using the High Performance Liquid chromatography (HPLC) procedure described by Rodriguez-Amaya and Kimura (2004). The β–carotene content was recorded as mg 100 g^{-1} on a fresh mass basis.

Table 1 Major traits of the sweetpotato progeny evaluated at five locations in Zambia, 2007/8 season.

		Traits*							
Genotype¤	ID[a]	Root shape	Colour Chart	Predominant skin Colour	Flowering Habit	Root dry mass (%)	Cracked roots (score)*	Sprouting (score)*	Weevil damage (score)*
L7- Chingovw a/36	G1	obvate	RHS:9/2 1355U	brownish orange	none	35.18	1	1	2
L7-W-119/107	G2	elliptic	RHS:9 137U	purple	none	36.98	1	1	2
L7-W-119/13	G3	elliptic	RHS 9/2 1355U	copper	moderate	39.34	1	1	2
L7- Chingovwa/84	G3	long elliptic	RHS:9/3 7507U	cream	sparse	36.7 3	1	1	2
L7- Chingovwa/62	G5	elliptic	RHS:9/2 1355U	brownish orange	profuse	34.98	1	1	1
L7- Excel/118	G6	long elliptic	RHS:9 137U	orange	none	37.66	1	1	2
L7- W119 -c/22	G7	obvate	RHS:9/1 1233U	pink	profuse	36.10	1	1	2
L7-199062.1/95	G8	elliptic	RHS:9/2 1 355U	copper	moderate	35.65	1	1	3
L7-15/1/17	G9	obvate	RHS:9 137U	orange	none	35.15	1	1	3
L7- Chingovwa/55	G10	obvate	RHS:9 137U	copper	profuse	41.46	1	1	2
L7- Chingovwa -c/24	G11	elliptic	RHS:9/3 750U	cream	moderate	36.25	1	1	2
L7- Chingovwa/83	G12	obva te	RHS:9/3 7507U	brownish orange	sparse	36.55	1	1	1
L7-W-119/89	G13	round	RHS 9/2 1355U	copper	profuse	35.95	1	1	2
L7- Chingovwa -c/56	G14	round	RHS:9/2 1355U	copper	moderate	37.95	2	1	3
L7- W119 -c/65	G15	elliptic	RHS:9 137U	copper	moderate	35.60	1	1	2

[a]ID = identification code for each genotype; **Scores for mole damage, weevil damage, and cracking were as follows: 1 = No symptom, 2 = 1-5 roots affected in a plot of 20 plants, 3 = any roots affected slightly (5-10% of root area), 4 = All roots affected moderately (11 - 25% of root area), and 5 = All roots affected severely (>25% of root area). ¤L7 = Luapula 2007 - meaning a selection done in Luapula Province in 2007

Data analysis

Each location in a given season was consider-ed as an individual environment and assigned a code as follows:

Environmental code	Location	Season
E1	Mansa -Mufulira	2007/2008
E2	Mutanda West	2007/2008
E3	Mansa -Main	2008/2009
E4	Mutanda East	2008/2009
E5	GART	2008/2009

Data were initially analyzed by conducting a separate ANOVA for each of the five environments using Genstat version 11.1 (Payne et al., 2007). Bartlett's (1937) and Levene's (1960) tests indicated homogeneity of error variances across environments and therefore data were pooled for the combined ANOVA across environments. Data was not transformed since there were no extreme values to warrant transformation.

Combined ANOVA across environments, basic rank and Spearman's rank correlation analyses on non-standardized data were conducted using Genstat version 11.1 (Payne et al., 2007). Stability analysis was performed on standardized data using the Additive main effect and Multiplicative Interaction (AMMI) model as described by Gauch and Furnas (1991). This model is more efficient than other methods in determining the most stable and high yielding genotypes in multi-environment trials (Manrique and Hermann, 2002). The model uses ANOVA to partition the Treatment sum of squares (SS) into the main effect SS for genotypes and environments, and the interaction SS for genotype x environment. The model then applies an Interaction Principal Component Analysis (IPCA) to determine pattern in the genotype x environment interaction means (Egesi and Asiedu, 2002). By plotting the main effects on the abscissa and the scores of the IPCA axes on the ordinate of a graph, the AMMI analysis provides a graphical representation (biplot) of the patterns represented by the specific interaction between genotypes and environments while simultaneously accounting for mean performance. The AMMI procedure in Genstat version 11.1 also ranks the top four genotypes in each environment.

Results

Genotype by Environment analyses of five traits

β-carotene content

The mean β-carotene content of the 15 polycross progeny was >4 mg 100 g^{-1} across environments (Table 2). Genotype G3 had the highest mean β-carotene content of 9.4 mg 100 g^{-1} across environments whereas G13 was the lowest. The highest mean β-carotene content across genotypes in an environment was recorded at E2 (6.2 mg 100 g^{-1}), followed by E3 (4.6 mg 100 g^{-1}). The E5 environment had the lowest mean β-carotene content (4.3 mg 100 g^{-1}) (Table 2). The highest β-carotene content was recorded at environment E2 for genotype G5 (11.3 mg 100 g^{-1}).

The main effect for G, and the G x E interaction were highly significant ($p<0.001$ and $p<0.01$, respectively) for β-carotene content (Table 5.3). The first interaction principal component (IPCA1) and the second (IPCA2) axes accounted for 86.8 and 9.6%, respectively, of the G x E sum of squares (SS) (Table 3).

Genotypes G1, G2, G4, G6, G8, G10, G11, and G12 exhibited IPCA1 values close to zero and above mean performance (>4.8 mg 100 g^{-1}) (Figure 1). Genotypes G5, G11, G10, G4, G12, and G1 performed best in environments E3 , G4, G10, G1, G2, G5, and G12 in E4, and G4, G5, G10, G1, G11, and G10 in E5 (Table 4). Genotype G14 was stable across all five environments but had low β-carotene content. Genotype G3 had the highest β-carotene content and since it was specifically adapted to three environments (E3, E4, and E5) and had an IPCA1 score of -2.63 it could be classified as unstable. Genotype G5 was the second highest performer across environments and was the highest in environment E1 and E2 (Tables 2 and 4; Figure 1). Genotype G13 (0.08 mg 100 g^{-1}) and G7 (0.16 mg 100 g^{-1}) recorded the lowest β-carotene content across environments (Table 2) and ranked among the lowest performing genotypes in each environment (Table 4). Environments E2 and E1 were unstable with IPCA1 scores of -4.61 and 1.78, respectively (Figure 1).

Table 2 mean β-carotene content (mg 100g^{-1}), root dry mass (%), harvest index, vine yield (t ha^{-1}) and root fresh yield (t ha^{-1}) of 15 genotypes of sweetpotato evaluated at five environments in Zambia

Genotypes	Mean across five environments				
	β–carotene content (mg 100 g^{-1})	Root dry mass (%)	Harvest index	Vine yield (t ha^{-1})	Root fresh yield (tha^{-1})
G1	6.508	33.94	0.74 4	3.097	8.62
G2	4.957	36.63	0.714	6.418	14.24
G3	9.421	35.47	0.781	5.428	14.72
G4	6.429	28.31	0.802	2.968	10.99
G5	8.428	25.15	0.800	3.032	10.79
G6	4.721	37.04	0.658	7.554	12.96
G7	0.165	33.45	0.72 5	4.764	11.27
G8	4.707	34.97	0.69 8	6.867	14.37
G9	1.116	42.07	0.785	4.240	13.97
G10	6.461	25.96	0.865	2.589	15.02
G11	6.537	33.41	0.812	4.081	14.05
G12	5.189	33.35	0.70 7	4.111	7.94
G13	0.086	37.90	0.787	5.472	17.47
G14	2.819	39.01	0.642	5.782	9.66
G15	4.701	30.50	0.81 9	3.084	11.2 9
Mean	4.816	33.81	0.756	4.632	12.49
SE(±)	0.722	1.95	0.041	1.382	2.39
Environment * means					
E1	4.592	34.99	0.803	2.639	11.27
E2	6.196	34.18	0.811	1.722	7.50
E3	4.597	33.10	0.724	4.706	12.86
E4	4.399	33.68	0.79 4	2.609	10.42
E5	4.299	33.10	0.647	11.486	20.41
Mean	4.816	33.81	0.756	4.632	12.49
SE(±)	0.153	0.42	0.010	0.748	0.72

* E1 = Mansa-Mufulira, E2 = Mutanda West, E3 = Mansa-Main, E4 = Mutanda East, E5 = GART;

Table 3: AMMI mean squares for b-carotene content (mg 100g-1) of 15 genotypes of sweetpotato evaluated at five environments in Zambia

Source	df	SS[a]	Mean squares	F value	Probability	% SS of: Treatment	G x E
Treatments	74	7579	102.42	102.33	0.00000	100.00	
Genotypes (G)	14	4932	352.27	351.97	0.00000	65.07	
Environment (E)	4	0	0.00	0.00	1.00000	0.00	
G x E	56	2647	47.27	47.23	0.00000	34.92	
IPCA 1	17	2298	135.20	135.08	0.00000		86.82
IPCA 2	15	253	16.90	16.88	0.00000		9.56
Residual	24	95	3.97	3.96	0.00008		3.59
Error	138	138	1.00				
Total	224	7731	34.51				

[a]SS = Sum of squares

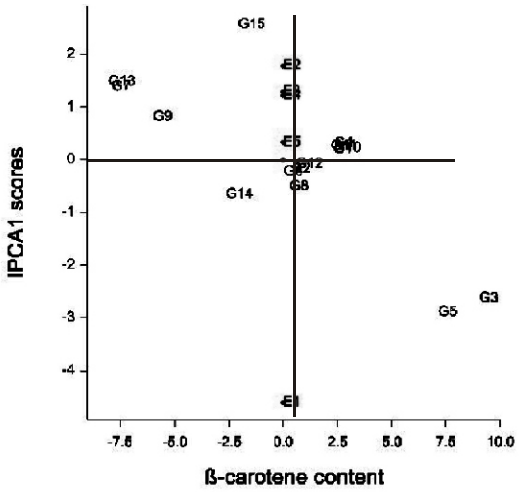

Figure 1 Biplot of mean β– carotene content (mg 100 g^{-1}) versus IPCA1 scoresfor 15 genotypes evaluated in five environments in Zambia. Grand mean = 4.82 mg 100 g^{-1}. Data standardized.

Table 4 Genotypes ranked per environment on the basis of mean β-carotene content. Environments ranked by IPCA1 score for β-carotene content (mg 100 g^{-1})

Genotype rank	Environments*				
	E1	E2	E3	E4	E5
1	G5	G5	G3	G3	G3
2	G3	G11	G5	G4	G4
3	G8	G15	G11	G10	G5
4	G10	G1	G1	G1	G10
5	G11	G10	G10	G2	G1
6	G6	G3	G4	G5	G11
7	G4	G4	G12	G1 2	G2
8	G2	G12	G15	G8	G12
9	G12	G6	G2	G11	G8
10	G1	G2	G6	G6	G6
11	G14	G8	G8	G15	G15
12	G9	G14	G14	G9	G14
13	G7	G9	G9	G14	G9
14	G15	G7	G7	G13	G13
15	G13	G13	G13	G7	G7
Mean[a]	0.005	-0.004	-0.003	-0.001	-0.001
Environment rank and (IPCA 1 score)	5 (-4.61)	1 (1.78)	2 (1.29)	3 (1.22)	4 (0.32)

* E1 = Mansa-Mufulira, E2 = Mutanda West, E3 = Mansa-Main, E4 = Mutanda East, E5 = GART;
[a]Means generated from standardized data

Root dry mass

The genotypes had a mean RDM of 33.1%. Genotype G14 had the highest RDM of 42.1% across environments. The highest mean RDM for all genotypes was recorded at E1 (35.0%), followed by E2 (34.2%). The E5 and E3 environments had the lowest mean RDM for all genotypes (33.1%) (Table 2).

The main effect for G, and the G x E interaction were highly significant ($p<0.001$) for RDM. The IPCA1 and IPCA2 axes explained 57.4% and 20.4%, respectively of the total G x E SS. Both IPCA1 and IPCA2 mean squares were highly significant ($p<0.001$ and $p<0.01$, respectively) (Table 5). The most stable genotypes for RDM with IPCA1 scores close to zero were G2, G3, G6, G8, G13, G14, and G9 (Figure 2; Table 6). Genotype G9 had the highest mean RDM (42.1%) across environments. The genotypes that performed below average across environments were also unstable (G15, G4, and G10). Genotype G5 recorded the lowest RDM (25.2%) across environments (Table 6) and was stable (IPCA = 0.11). All the environments performed similarly (range of 1.89%) but E2 (IPCA1 = -1.82) was the most unstable environment. Environments E4 (IPCA1 = -0.25) and E5 (IPCA1 = 0.27) were stable for RDM (Figure 2).

Table 5 AMMI mean squares for root dry mass (%) of 15 genotypes of sweetpotato evaluated at five environments in Zambia, 2008/2009.

Source	df	SS	Mean squares	F value	Probabi lity	% SS of: Treatment	G x E
Treatments	74	1462.3	19.76	19.77	0.00000	100.00	
Genotypes (G)	14	1301.3	92.95	93.00	0.00000	88.99	
Environments (E)	4	0.0	0.00	0.00	1.00000	0.00	
G x E	56	161.0	2.88	2.88	0.00005	11.01	
IPCA 1	17	92.5	5.44	5.45	0.00000		57.45
IPCA 2	15	32.8	2.19	2.19	0.00938		20.37
Residual	24	35.7	1.49	1.49	0.08151		22.17
Error	138	137.9	1.00				
Total	224	1608.1	7.18				

[a]SS = Sum of squares

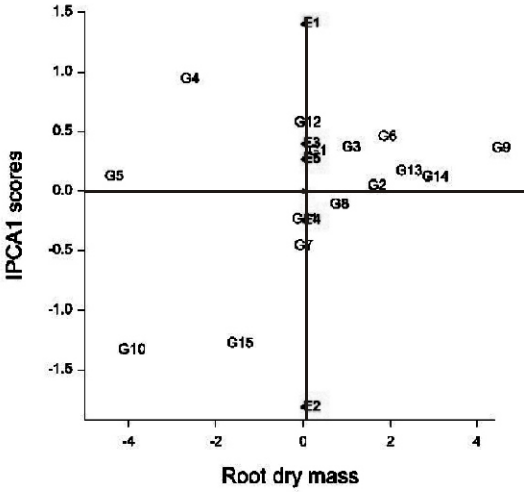

Figure 2 Biplot of mean root dry mass (%) and IPCA1 scores of 15 genotypes planted at five locations in Zambia. Grand mean = 33.8%. Data standardized.

Table 6 Genotypes ranked per environment on the basis of mean root dry mass. Environments ranked by IPCA1 score for root dry mass (%)

Genotype rank	Environments* E1	E2	E3	E4	E5
1	G9	G9	G9	G9	G9
2	G14	G14	G14	G14	G14
3	G6	G13	G13	G13	G13
4	G13	G15	G6	G2	G2
5	G2	G2	G2	G6	G6
6	G3	G6	G3	G3	G3
7	G8	G7	G8	G11	G11
8	G1	G8	G1	G8	G8
9	G12	G3	G12	G1	G1
10	G7	G1	G11	G12	G12
11	G4	G11	G7	G7	G7
12	G11	G12	G4	G15	G15
13	G15	G10	G15	G4	G4
14	G5	G4	G5	G10	G10
15	G10	G5	G10	G5	G5
Mean[a]	-0.0007	0.0006	-0.0012	0.0019	0.0027
Environment rank and (IPCA 1 score)	1 (1.40)	5 (-1.82)	2 (0.40)	3 (-0.25)	4 (0.27)

*E1 = Mansa-Mufulira, E2 = Mutanda West, E3 = Mansa-Main, E4 = Mutanda East, E5 = GART;
[a]Means generated from standardized data

Harvest index

The genotypes had a mean HI of 0.756. Genotype G10 had the highest HI of 0.865 across environments. The lowest mean HI was recorded for G14 (0.642) across environments. The highest mean HI was calculated at environment E2 (0.811), followed by E1 in the same year (0.803). The E5 environment had the lowest mean HI (0.647) (Table 2).

The G and the G x E were highly significant ($p<0.001$). The IPCA1 and the IPCA2 mean squares were both highly significant ($p<0.001$) and their SSs accounted for 75.6% and 15.1%, respectively, of the G x E SS (Table 7). The most stable (IPCA1 scores close to zero) genotypes for HI with above average performance (>0.75) were G10, G15, G11, G4, G5, G13, and G9 and ranked highly across environments. Another set of genotypes, G2, G12, G7, G8, and G6 with IPCA1 scores close to zero, had below average HI and their ranks were low across the environments (Figure 3; Table 8). Genotype G10 was stable across the five environments and had the highest mean HI (0.86) across environments. Genotype G14 and G6 had the lowest mean HI (0.66 and 0.64, respectively) across environments but G6 was stable (IPCA1 = 0.07) whereas G14 was unstable (IPCA1 = -2.4). Environment E1 was the most stable (IPCA1 = -0.52) for all the genotypes followed by E4 (IPCA1 = 0.9) (Table 8).

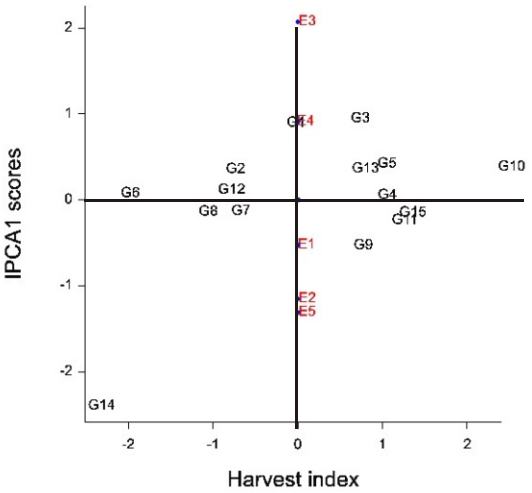

Figure 3 Biplot of mean harvest index and IPCA1 scores of 15 genotypes evaluated in five environments in Zambia. Grand mean = 0.76. Data standardized.

Table 7 AMMI mean squares for harvest index of 15 genotypes of sweetpotato evaluated at five environments in Zambia, 2008/2009.

Source	df	SS[a]	Mean squares	F value	Probability	% SS of:	
						Treatment	G x E
Treatments	74	662.8	8.956	10.84	0.00000	100.00	
Genotypes (G)	14	380.1	27.153	32.85	0.00000	57.35	
Environments (E)	4	0.0	0.003	0.00	0.99 997	0.00	
G x E	56	282.6	5.047	6.11	0.00000	42.64	
IPCA 1	17	213.6	12.567	15.21	0.00000		75.58
IPCA 2	15	42.7	2.847	3.44	0.00003		15.11
Residual	24	26.3	1.095	1.32	0.15903		9.31
Error	138	114.1	0.826				
Total	224	783.3	3.497				

[a]SS = Sum of squares

Table 8 Genotypes ranked per environment on the basis of mean harvest index. Environments ranked by IPCA1 score for harvest index

Genotype rank	Environments*				
	E1	E2	E3	E4	E5
1	G15	G15	G10	G10	G10
2	G10	G10	G3	G15	G4
3	G11	G11	G5	G5	G11
4	G13	G9	G1	G11	G9
5	G9	G4	G4	G13	G14
6	G5	G13	G13	G3	G5
7	G4	G5	G11	G4	G15
8	G7	G7	G2	G9	G13
9	G3	G14	G15	G1	G3
10	G1	G3	G12	G7	G8
11	G12	G8	G9	G2	G12
12	G2	G12	G8	G12	G2
13	G8	G1	G7	G8	G1
14	G6	G2	G6	G6	G7
15	G14	G6	G14	G14	G6
Mean[a]	0.001	0.008	0.008	-0.010	0.006
Environment rank and (IPCA 1 score)	3 (-0.52)	4 (-1.15)	1 (2.07)	2 (0.91)	5 (-1.31)

*E1 = Mansa-Mufulira, E2 = Mutanda West, E3 = Mansa-Main, E4 = Mutanda East, E5 = GART;
[a]Means generated from standardized data

Vine fresh yield

The genotypes had a mean VFY of 4.6 t ha-1. Genotype G6 had the highest mean VFY of 7.5 t ha-1 across environments. The highest mean (11.49 t ha-1) vine fresh yield across genotypes, however, was recorded at environment E5, followed by environment E3 (4.71 t ha-1). The mean (11.5 t ha-1) VFY for Environment E5 was more than double the mean (4.7 t ha-1) VFY of E3 and five times more than the other environments (Table 2).

The G x E was highly significant ($p<0.001$). The IPCA1 and the IPCA2 accounted for 55% and 22.5%, respectively, of the G x E SS (Table 9). Genotype G7 was the most stable (IPCA1 = 0.13) combined with above average mean performance (4.76 t ha-1). Other stable genotypes with high mean VFY were G13 and G8 (5.47 and 6.87 t ha-1, respectively). Genotype G2 and G6 performed above average but were less stable. There were more stable genotypes combined with below average mean performance (<4.6 t ha-1); for example: G7, G9, G4, G11, and G14 (Table 10). In terms of environments, E1 was most stable (IPCA1 = -0.16). Environment E2 was a high yielding environment but was most unstable (Figure 4; Table 10). Genotype G6 was the best performing in all environments except in E3 where it ranked third (Table 10).

Table 9 AMMI mean squares for vine fresh yield (t ha-1) of 15 genotypes of sweetpotato evaluated in five environments in Zambia, 2008/2009.

Source	df		Mean squares	F value	Probability	% SS of:	
						Treatment	G x E
Treatments	74	849.1	11.47	14.33	0.00000	100.00	
Genotypes (G)	14	254.8	18.20	22.73	0.00000	30.01	
Environments (E)	4	403.3	100.82	38.17	0.00000	47.50	
G x E	56	191.1	3.41	4.26	0.00000	22.50	
IPCA 1	17	105.1	6.18	7.72	0.00000		55.00
IPCA 2	15	43.0	2.86	3.58	0.00003		22.50
Residual	24	43.0	1.79	2.24	0.00198		22.50
Error	138	110.5	0.80				
Total	224	968.0	4.40	19.00			

[a]SS = Sum of squares

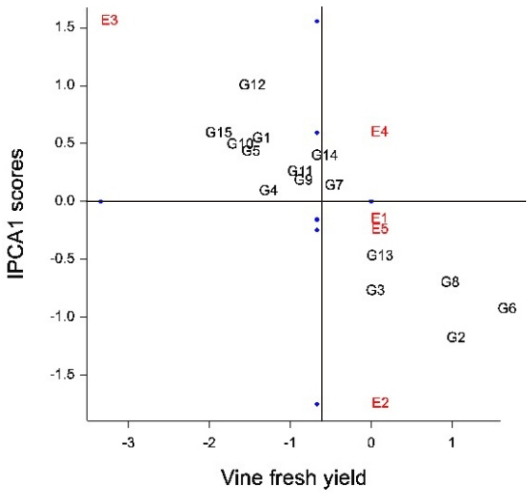

Figure 4 Biplot of mean vine fresh yield (t ha-1) and IPCA1 scores of 15 genotypes planted at five locations in Zambia. Grand mean = 4.6 t ha-1. Data standardized

Table 10 Genotypes ranked per environment on the basis of mean vine fresh yield (t ha-1). Environments ranked by IPCA1 score for vine fresh mass (t ha-1)

Genotype rank	Environments*				
	E1	E2	E3	E4	E5
1	G6	G6	G8	G6	G6
2	G8	G2	G14	G8	G3
3	G2	G8	G6	G2	G2
4	G13	G3	G13	G7	G8
5	G7	G13	G7	G13	G14
6	G14	G7	G2	G14	G13
7	G9	G11	G9	G9	G11
8	G3	G9	G12	G4	G12
9	G4	G1	G4	G1	G7
10	G1	G4	G1	G3	G9
11	G11	G10	G15	G5	G15
12	G12	G5	G3	G11	G1
13	G5	G14	G5	G10	G5
14	G10	G12	G11	G12	G10
15	G15	G15	G10	G15	G4
Mean[a]	-0.001	0.007	-3.346	-0.002	0.0001
Environment rank and (IPCA 1 score)	3 (-0.16)	5 (-1.75)	1 (1.56)	2 (0.59)	4 (-0.24)

* E1 = Mansa-Mufulira, E2 = Mutanda West, E3 = Mansa-Main, E4 = Mutanda East, E5 = GART;
[a]Means generated from standardized data

Root fresh yield

The genotypes had a mean RFY of 12.5 t ha-1. Genotype G13 had the highest RFY with a mean of 17.5 t ha-1 across environments. The E5 environment had the highest mean RFY (20.4 t ha-1) across genotypes while environment E2 had the lowest mean RFY (7.5 t ha-1) (Table 2).

The G main effect and the G x E were highly significant ($p<0.001$) (Table 11). The IPCA1 and the IPCA2 accounted for 47.3% and 37.8%, respectively, of the G x E SS and were highly significant ($p<0.001$) (Table 11). The most stable genotypes with IPCA1 scores close to zero combined with above average performance (>12.5 t ha-1) across environments were G6, G10, G9, G8, and G2 (Figure 5). Genotype G13 was the highest yielding but was less stable (IPCA1 = 0.95). It did not perform very well in environment E5, though it was the best performing genotype in the rest of the environments. Among the low yielding genotypes, G12 and G15 with IPCA1 scores of 0.1 and -0.39, respectively, were the most stable (Figure 5 and Table 12). Environment E2 was the most stable environment (IPCA1 = 0.27) but had the lowest mean (7.5 t ha-1) yield across genotypes. Conversely, environment E5 was very unstable (IPCA1 = -2.58) (Figure 5).

Table 11 AMMI mean squares root fresh yield (t ha-1) of 15 genotypes of sweetpotato evaluated at five environments in Zambia

Source	df		Mean squares	F value	Probability	% SS of:	
						Treatment	G x E
Treatments	74	884.7	11.956	11.97	0.00000	100.00	
Genotypes (G)	14	399.1	28.510	28.53	0.00000	45.11	
Environment (E)	4	0.0	0.000	0.00	1.00000	0.00	
G x E	56	485.6	8.671	8.68	0.00000	54.89	
IPCA 1	17	229.7	13.511	13.52	0.00000		47.30
IPCA 2	15	183.3	12.221	12.23	0.00000		37.75
Residual	24	72.6	3.025	3.03	0.00003		14.95
Error	138	13.9	0.999				
Total	224	1051.7	4.695				

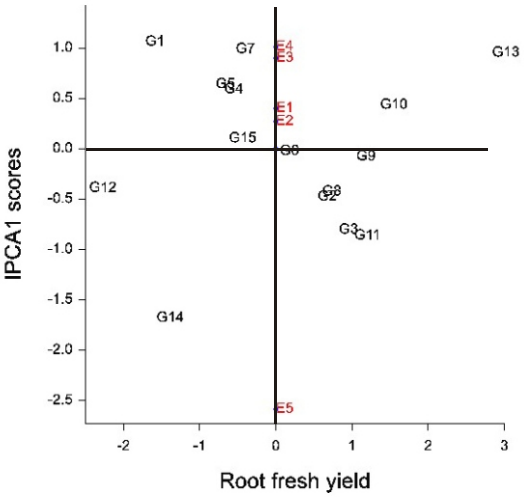

Figure 5 Biplot of mean root fresh yield (t ha-1) and IPCA1 scores of 15 genotypes planted at five locations in Zambia. Grand mean = 12.5 t ha-1. Data standardized.

Table 12 Genotypes ranked per environment on the basis of mean performance. Environments ranked by IPCA1 score for root fresh yield (t ha-1)

Genotype rank	Environments*				
	E1	E2	E3	E4	E5
1	G13	G13	G13	G13	G3
2	G7	G9	G10	G10	G11
3	G9	G11	G3	G3	G14
4	G1 5	G10	G8	G8	G8
5	G10	G7	G1	G2	G2
6	G11	G2	G2	G6	G9
7	G6	G8	G4	G9	G13
8	G2	G15	G6	G4	G10
9	G5	G6	G5	G7	G6
10	G8	G3	G7	G5	G12
11	G4	G5	G9	G11	G15
12	G3	G4	G12	G1	G4
13	G1	G14	G11	G15	G5
14	G14	G1	G15	G12	G7
15	G12	G12	G14	G14	G1
Mean[a]	0.001	-0.003	0.0001	0.001	-0.0001
Environment rank and (IPCA 1 score)	3 (0.40)	4 (0.27)	2 (0.90)	1 (1.01)	5(-2.58)

*E1 = Mansa-Mufulira, E2 = Mutanda West, E3 = Mansa-Main, E4 = Mutanda East, E5 = GART;
[a]Means generated from standardized data

Correlations among five traits

Root dry mass was negatively correlated with β-carotene content, VFY, and RFY whereas it was positively correlated with HI. β-carotene content was negatively correlated with HI and positively correlated with VFY. HI was negatively correlated with both VFY and RFY while VFY and RFY were positively correlated (Table 13)

Table 13: Phenotypic correlations among five traits measured on 15 genotypes in five environments

	Traits				
Traits	Root dry mass (%)	β-carotene content (mg 100g^{-1})	Harvest index	Vine fresh yield (t ha^{-1})	Root fresh yield (tha^{-1})
Root dry mass (%)	-				
-carotene content (mgl00g^{-1})	-0.404***	-			
Harvest index	0.223***	-0.234***	-		
Vine fresh yield (tha^{-1})	-0.172*	0.162 *	-0. 687***	-	
Root fresh yield (tha^{-1})	-0.152*	0.033 NS	-0.13 6*	0.739***	-

***Significant at p<0.05, 0.001, respectively.
NS=Not Significant.

Discussions and Conclusion

The mean squares for the G x E were highly significant (p<0.001) for β-carotene content, RDM, HI, VFY, and RFY, indicating differential response of genotypes relative to each other across the five environments. The G x E interactions are revealed by the changes in the rank order of the genotypes across the environments for the five traits (Tables 4, 6, 8, and 10). The AMMI analysis identified genotypes that were stable across environments and these are discussed for each trait.

Spearman's rank correlations between environments were positive and high for β-carotene content, RDM, HI, and VFY (Tables 13, 15, 17, and 19; , respectively). These correlations can be attributed to a number of genotypes maintaining consistent rankings across environments. For example, for the trait RDM, genotypes G9, G14, and G13 had consistently high values while others, G10, G4, and G5, had consistently low values (Table 6).

Mean β-carotene content ranged from 0.09 to 9.4 mg 100 g-1 indicating that β-carotene content was highly variable among the genotypes. The mean RDM (33.8%) of the 15 genotypes was high relative to the popular local, cultivar with genotype G9 recording the highest mean RDM of 42%. The mean HI was above 50% for all the genotypes indicating that most of the photosynthates were partitioned to the roots. Genotype G6 and G8 had the highest VFY and may be considered either as a vegetable or livestock feed depending on their palatability. The mean RFY ranged from 7.9 to 17.5 t ha-1. The overall mean RFY of the 15 progeny selected from the polycross was 12.5 t ha-1 and was higher than the average of the germplasm collected (detailed in Chapter 3) which was 8.9 t ha-1. This is a remarkable increase in yield for this polycross derived set of genotypes.

β-carotene content

The subdivision of G x E for β-carotene content in roots indicated that the first two IPCA axes accounted for 96% of the total variability. However, the high G (65% of treatment SS) and the relatively low G x E (35% of treatment SS) for β-carotene content may indicate that the evaluation for high, stable performance can be done using well chosen environments. This result concurs with that of Grûneberg et al. (2005) and Manrique and Hermann (2002) who reported the SS for G x E for β-carotene smaller than that for the main effects of genotype. Similar results were obtained for cassava for total carotenoids (Ssemakula et al., 2007). The relatively high stability of the genotypes for β-carotene content may indicate that this trait is less influenced by the environment than, for example, RFY. This suggests that prospects for improving β-carotene content in sweetpotato are favourable. Eight genotypes, namely G1, G2, G4, G6, G8, G10, G11, and G12 performed above average and were stable across environments. Genotypes with high β-carotene can be identified early in the breeding programme and a few, well chosen environments can be used. For example, genotype G3 was the best performer and was best adapted to three environments, E3 (8.9 mg 100 g-1), E4 (10.4 mg 100 g-1), and E5 (9.8 mg 100 g-1), but its performance was lower in E2 (7.5 mg 100 g-1).

Root dry mass composition

Root dry mass is a very important trait for consumers in Zambia. The 15 selected polycross progeny recorded RDM above 30% which is the preferred level among consumers. This is an indication that the objective of breeding high β-carotene and high RDM genotypes is achievable. For example, genotypes G2, G6, and G8 were stable across all five environments with above average performance for both β-carotene content (5.0, 4.7, and 4.7 mg 100 g-1, respectively) and RDM (37, 37, and 35%, respectively). These three

genotypes can consequently be recommended for all five environments (Figure 5). Genotype G3, which had the highest mean β-carotene level of 9.4 mg 100 g-1, was, however, more stable for RDM with above average performance (35%) (Figure 2). Genotype 3 had the third highest mean yield across environments (14.7 t ha-1). It was the top performer in environment E5 (30 t ha-1) and is therefore recommended for this specific environment.

Harvest index

Grûneberg et al. (2005) found that genotypes with high yield and high yield stability tend to also have high HI and high HI stability. In this study, only two genotypes, G9 and G10, conformed to this finding (Figures 3 and 5). Therefore, an ideal genotype will need to balance the allocation of photosynthates between the development of harvestable roots and adequate vine production.

Genotype G6 had the highest VFY (7.6 t ha-1) across environments and its mean HI (0.66) was low and stable but the RFY was average. The genotype can be considered for forage production for livestock or for vegetable production depending on the palatability. In addition, it provides sufficient quantities of vines for propagation.

Root fresh yield

The significant ($p<0.001$) G x E mean square and its high relative proportion of Treatment SS (55%) for RFY is expected because yield is a polygenic trait (Easwari and Sheela, 1998; Cach et al., 2006) and, therefore, influenced by the environment (Table 11). Other G x E studies (Collins, et al., 1987; Bacusmo et al., 1998; Naskar and Singh, 1992; Ngeve, 1993; Manrique and Hermann, 2002; Grûneberg et al., 2005) have reported that in sweetpotato, RFY is sensitive to G x E. The strong influence of the environment on RFY makes the potential genetic gain in RFY unpredictable. Hence, early testing of genotypes in multi-locations to identify those with specific versus general stability is necessary. The G x E (55% of Treatment SS) for root fresh yield was larger than the G (45% of Treatment SS) main effect. This implies that higher yields could be attained by improving crop management practices in environments suited to the crop besides emphasising the improvement of genotypes.

Correlations among five traits

Negative correlation between RDM and β-carotene content confirmed previous results (Hernandez et al. 1967 and Jones, 1977). RDM was also negatively correlated with VFY and RFY indicating that selecting for higher RDM may compromise the yield of both the roots and the vines. There were positive associations among VFY and RFY suggesting that breeding for any of these traits would not reduce the desired level of the other.

General conclusion

The magnitude of the G x E for β-carotene content, RDM, and HI was small and selection for these traits may be conducted in a few, well selected environments. Conversely, RFY and VFY yield may require early testing in varied environments to select genotypes with either wide or specific adaptation. It can be concluded that it is possible to breed for high β-carotene, high RDM and high yield sweetpotato genotypes with wide or specific adaptation in Zambia as the AMMI analysis identified genotypes G2, G6, and G8 as stable across environments for both β-carotene content and RDM. They performed above average for both traits. Therefore, G2, G6, and G8 qualify as genotypes with above average yield that would do well in all the environments with acceptable β-carotene content and RDM. Genotype G3 was best suited for environment E3, E4, and E5 and had the highest mean β-carotene content (9.4 mg 100 g-1), and high mean RDM (35.5%), and high mean RFY of 14.7 t ha-1 across the environments. Also it had above average mean RFY (14.7 t ha-1) meeting the basic criteria for a genotype preferred by consumers (as determined in the PRA study detailed in Chapter 2). These identified genotypes will undergo further evaluation that may culminate in their release for production by Zambian farmers.

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Appendices

Appendix 1 Number of seed produced from each parental genotype in two polycrosses (12 x 12,and 30 x 8) conducted at Mansa Research Station.

ID	Parent	Source	Number of seeds	
			12 parent	30 parent
1	Excel	CIP Kenya	2,395	
2	Kabalenge	Local	292	544
3	Matembele 3K	Local	221	18
4	W-119	CIP Kenya	3,095	888
5	L2-4/20/5	Local	268	54
6	15/1	Local	1,320	280
7	Unknown 2/1	Local	1,670	32
8	L3-199084/1	Local	2,822	1,500
9	No name 13K	Local		272
10	Zambezi/1	Local		112
11	Kakamega	CIP Kenya		3,380
12	L3-L0-4/10/6	Local		234
13	Munwe umo	Local		228
14	Carrots Mwewa	Local		3,696
15	Kasompe	Local		50
16	No name 14N	Local		76
17	Katansha	Local		1014
18	Zambezi	Local		934
19	L3-Mugamba 3/1	Local		104
20	Lukusashi	Local		56
21	199047/4	Local		22
22	Carrot -C	Local		3,476
23	Resisto	CIP Kenya		1066
24	1998 -12 -3	ARC - VOPI South Africa		2,715
25	1999 -1 -7	ARC - VOPI South Africa		1,738

Appendix 2 Soil nutrient analysis of the five experimental sites for the G x E trial

Environment	Designation	pH	Soil nutrients*											
			P	Ca	Mg	Na	K	Cu	Zn	Mn	Fe	% N	% C	CEC
Mansa -	E1	4.6	4	25	22	-	5	7	trace	22.2	39.1	0.11	1.5	-
Mufulira Mutanda -	E2	4.3	11	32	10	-	17	-	1.8	-	16.7	-	0.88	-
West Mansa -	E3	4.1	10	53	19	2.2	26.8	0.4	2.5	12	41.4	0.8	1.17	3.84
Main Mutanda -	E4	4.3	7	85	19	2.1	24.6	1	1.3	23	42	0.8	1.08	2.44
East GART	E5	4.1	2	30	14.7	-	6	-		14.7		0.7	1.02	

*Units are ppm where not indicated