Journal of Tropical Agriculture 45 (1-2): 14-20, 2007

Stability of total carotenoid concentration and fresh yield of selected yellow-fleshed cassava (*Manihot esculenta* Crantz)

G. Ssemakula[1], A.G.O. Dixon, and B. Maziya-Dixon

International Institute of Tropical Agriculture (IITA), PMB 5320 Ibadan, Nigeria

Received 15 May 2007; received in revised form 7 July 2007; accepted 9 July 2007.

Abstract

The effects of genotype (G), environment (E), and G x E interaction on carotenoid concentration and yield of 25 yellow-fleshed (YF) and three white-fleshed (WF) cassava genotypes were investigated at five locations in Nigeria for two consecutive cropping seasons. The locations represented the major cassava growing agroecologies in the country and the objective was to identify stable cassava clones for yield and carotenoid levels. Location (L) and G main effects, and year (Y) x L and G x L interactions were significant (p<0.001) for fresh yield and total carotenoid concentrations; G x L was the largest component of G x E. AMMI analysis revealed that carotenoid concentration is a stable trait; while yield is relatively unstable. The most stable genotypes for total carotenoid levels above the overall mean were 01/1277, 01/1235, 01/1371, 01/1413, and 01/1442. Likewise, the most stable clones for yield with above average performance were 01/1235, 94/0006, and 01/1206. The high G and low E effects, and the relatively low GEI on total carotenoid concentration imply that evaluation and selection can be effectively done in fewer environments to distinguish clones with high and stable performance while yield requires early testing in diverse and multiple environments to identify genotypes with broad and specific adaptations. Our results suggest that it is possible to breed cassava with high and stable performance for both yield and carotenoid contents.

Keywords: Yellow-fleshed cassava, genotype by environment interaction, micronutrients.

Introduction

Both genotype and environment determine the phenotype of an individual. These two effects, however, are not always additive because of the interaction between genotype and environment (GEI). GEI is the result of inconsistent performances of genotypes across environments. A significant GEI results from changes in the magnitude of differences between genotypes in different environments or changes in the relative ranking of genotypes (Fernandez, 1991). Breeders face the GEI challenge by evaluating genotypes in several environments to ensure that they select accessions with high and stable performance over a wide range of environments. Genotypes whose GEI is insignificant are said to be stable.

The yellow flesh colour found in some cassava genotypes is associated with the density of micronutrients, such as ^{2}-carotene (Iglesias et al., 1997; Chávez et al., 2005). Therefore, yellow-fleshed (YF) cassava has been at the centre-stage in breeding for enhanced micronutrients in this crop, a staple in much of the poverty-stricken Africa. A breeding program for enhanced carotenoids in cassava was initiated at the International Institute of Tropical Agriculture (IITA), Nigeria in 2003 under the auspices of Harvest Plus, a challenge programme of the Consultative Group of International Agricultural Research (CGIAR). The starting point was the screening of YF cassava clones for carotenoid levels to establish useful variability for this trait. Twenty two clones were identified with relatively high levels of ^{2}-carotene and are currently being used in breeding. It is important that high and stable levels of carotenoids are incorporated into a good and stable agronomic background to enhance their

[1]Author for correspondence: Tel. 234 2 241 2626; Fax 234 2 241 2221; Email <g.ssemakula@cgiar.org>.

acceptability. High yield is an important factor that contributes to the acceptability of the new genotypes. This study, therefore, was designed to evaluate YF clones across locations for total carotenoid concentration and fresh yield, to determine the magnitude of genotype (G), environment (E), and GEI effects on these traits, and to identify stable and high performing clones.

Materials and methods

Twenty-five YF and three white-fleshed (WF) check clones of cassava (*Manihot esculenta* Crantz) were planted at five locations in Nigeria during 2004/2005 and 2005/2006. The locations, Mokwa, Ibadan, Ubiaja, Zaria, and Onne represent the major cassava growing agroecologies in the country. The clones were grown under rain-fed conditions in a randomized complete block design with four replications. Planting in both years was done in July, i.e., during the rainy season. Each plot consisted of 40 plants in four rows (ridges 0.3 m high and 10 m long) spaced 1 m apart. Spacing within the ridges was 1 m. No fertilizers or herbicides were applied during the course of the experiment; although weeding was done as deemed necessary. Harvesting in both years was done at approximately 12 months after planting (MAP). At harvest, the fresh tuberous root yield per plot was recorded. Only the inner plants in a plot were harvested (excluding the border rows).

Determination of total carotenoids

Three roots from each of the three plants per clone were randomly selected, washed in tap water, and air-dried on a clean concrete surface. The roots were peeled with a stainless steel knife and rinsed in deionised water. Thereafter, each root was cut into four longitudinal sections using a stainless steel knife. A quarter was removed from each root and chopped into small pieces. The chopped pieces from each root were mixed, and quickly rinsed in deionised water. All sampling were done under subdued light and samples were immersed in liquid nitrogen, and packed in polythene bags. All the samples were stored in a deep freezer at –80°C until they were analysed. Total carotenoid concentration was determined spectrophotometrically as described in the HarvestPlus Handbook for Carotenoid Analysis (Rodriguez-Amaya and Kimura, 2004).

Data analysis

The data were subjected to combined analyses of variance using the GLM procedure of Statistical Analysis System (SAS) to determine the magnitude of the main effects and interactions. The unbalanced data consisting of 27 genotypes and three replications, which were common to all the locations, were subjected to the Additive Main effect and Multiplicative Interaction (AMMI) analysis using Matmodel (Gauch and Furnas, 1991). The biplots [main effect means vs. first Interaction Principal Component Axis (IPCA1)] from the AMMI analysis were used to study the effects of G, E, and GEI. The biplots were also used to identify genotypes with broad or specific adaptation to target agroecologies or environments for yield and total carotenoid concentrations.

Results

Mean performance of the cassava genotypes across the environments is presented in Table 1. The clones had mean yields > 15 t ha^{-1}. YF clone 98/2132 gave the highest fresh yield of 26.1 t ha^{-1} across locations. The highest mean fresh yield, however, was recorded at Onne in 2005 (29.34 t ha^{-1}), followed by Ibadan in 2006 (23.83 t ha^{-1}), and Ibadan in 2005 (23.62 t ha^{-1}). The Zaria (2005) environment gave the lowest mean yield (7.26 t ha^{-1}). The highest mean total carotenoid concentration across locations was from the YF clone 01/1368 (7.34 ¼g g^{-1}) followed by 01/1663 (7.1 ¼g g^{-1}), 01/1371(6.92 ¼g g^{-1}), and 01/1412 (6.63 ¼g g^{-1}).

Combined analyses of variance using the GLM procedure of SAS (Table 2) indicated that location (L) and genotype (G) main effects were significant (p<0.001) for fresh yield and total carotenoid concentration. Year effect (Y) was also significant (p<0.001) for total carotenoid concentration but not for fresh yield. For both traits, Y x L and G x L interactions

Table 1. Mean yield and total carotenoid concentration of 24 yellow and three white-flesh cassava clones and evaluated at five locations in Nigeria, 2004/05-2005/06.

Clone	Fresh yield (t ha^{-1})	Total carotenoid concentration (μg g^{-1})
01/1115	13.93	5.20
01/1206	22.87	3.72
01/1224	14.68	6.04
01/1235	18.83	5.74
01/1273	10.58	5.47
01/1277	14.45	5.67
01/1331	8.2	5.8
01/1335	14.95	5.58
01/1368	19.33	7.34
01/1371	14.06	6.92
01/1380	18.24	3.47
01/1404	15.94	5.69
01/1412	22.35	6.63
01/1413	15.78	6.38
01/1442	15.78	5.54
01/1610	15.93	5.64
01/1646	17.52	3.96
01/1649	14.58	5.67
01/1663	15.71	7.10
90/01554	15.68	3.25
94/0006	19.92	3.08
94/0330	12.33	3.69
95/0379	19.31	4.34
98/2132	26.1	4.77
TME1 (check)	18.88	0.93
30572 (check)	15.41	0.91
91/02324 (check)	25.14	0.93
Mean	16.91	4.80
SE (±)	0.784	0.35
Environment means		
Ibadan2005	23.62	4.59
Ibadan2006	23.83	4.52
Mokwa2005	12.1	4.91
Mokwa2006	21.19	5.5
Onne2005	29.34	4.01
Onne2006	17.35	4.24
Ubiaja2005	11.89	4.53
Ubiaja2006	14.15	5.05
Zaria2005	7.26	4.73
Zaria2006	8.32	5.87
Mean	16.91	4.80
SE (±)	2.33	0.18

were significant, but G x Y interaction was not. The G x L x Y interaction was highly significant for fresh yield (p<0.001) and barely significant for total carotenoid concentration (p<0.05). G x L was the largest component of GEI for both these traits.

The relative magnitude of the main effects and their interactions for all traits measured as a proportion of the total sum of squares showed that G impacted more on total carotenoid concentration than the other effects and interactions. Likewise, L impacted more on fresh yield. G x Y effect for total carotenoid content was more than twice that of the fresh yield although both interactions were of relatively low magnitude.

The analysis of variance of the AMMI model (Table 3) for total carotenoids also showed that the effects of G, E, and GEI were significant (p<0.001). Environments (E) (obtained as L x Y combinations), genotypes, and GEI were highly significant (p< 0.001) for fresh yield and total carotenoid concentration. Genotypes accounted for 73.7% of the total sums of squares for total carotenoid concentration, while E accounted for just 6.6%, and GEI 14.9%. Genotypes, E, and GEI also accounted for 16.3%, 49.7%, and 14.9% of total sums of squares for yield respectively. Using the main effects and the first principal component axis (IPCA1) of the GEI, the AMMI analysis provides a graphical representation (biplot) to summarize information on the main effects and the first principal component scores of the GEI of both genotypes and environments simultaneously for fresh yield and total carotenoid concentrations (Fig. 1 and 2).

The postdictive success for AMMI, using all the available data and F-test at 0.05 probability level, involved the inclusion of the first four interaction PCA axes in the model for fresh yield and first six interaction PCA axes for total carotenoid concentration (Table 3). A significant feature of the multivariate models (including AMMI analysis) is that they account for a large proportion of the pattern related to treatment design in the first few dimensions. The subsequent dimensions account for diminishing proportion of this and an increasing percentage of noise. Since the

Table 2. Combined analyses of 25 yellow-fleshed and 3 white-fleshed cassava clones grown for two years (2005 and 2006) at five locations in Nigeria for total carotenoids content and fresh yield.

Source	DF	Type IIISS	Meansquare	F value	Pr > F	% of totalsum of Squares
Total carotenoid concentration						
Rep (Year*Location)	10	6.125	0.6125	0.91	0.5272	
Year (Y)	1	28.28	28.28	46.18	<.0001	1.2
Location (L)	4	103.62	25.91	42.3	<.0001	4.3
Y*L	4	22.28	5.57	9.09	0.0023	0.9
Genotype (G)	27	1679.85	62.22	48.36	<.0001	70
Y*G	27	34.73	1.29	1.45	0.0959	1.4
L*G	108	224.38	2.08	3.08	<.0001	9.3
Y*L*G	102	90.62	0.89	1.32	0.0433	3.8
Error	260	175.57	0.68			
Total	543	2400.06				
Fresh yield:						
Rep (Year*Location)	30	2343.32	78.11	3.03	<.0001	
Year (Y)	1	56.7	56.7	0.73	0.401	0.1
Location (L)	4	29975.39	7493.85	95.94	<.0001	28.4
Y*L	4	12990.61	3247.65	41.58	<.0001	12.3
Genotype (G)	27	16561.86	613.4	24.44	<.0001	15.7
Y*G	27	677.63	25.1	0.52	0.9743	0.6
L*G	108	11629.41	107.68	4.17	<.0001	11
Y*L*G	102	4952.3	48.55	1.88	<.0001	4.7
Error	751	19374.37	25.8			
Total	1054	105559.8				

AMMI1 biplots captured a large proportion of the pattern in the data (89%, and 86% of the treatment sum of squares for total carotenoid concentration and fresh yield respectively), they are accurate enough to explain the main effects and the pattern of GEI.

Displacement along the x-axis of the biplots reflected differences in main effects, whereas displacement along the y-axis exhibited differences in the interaction effects. Genotypes with IPCA1 scores near zero had little interaction with environments. Genotypes or environments on the same parallel line relative to the y-axis have similar mean values for the trait, and a genotype or environment on the right side of the midpoint of this axis has higher mean values than those on the left hand side.

The most stable clones for total carotenoid concentration with above average performance were 01/1235, 01/1277, 01/1404, 01/1413, 01/1371, and 01/1442 (Fig. 1). The clones performed best in the Ubiaja 2006 environment. Clones 01/1646, 01/01554, and 01/1206 were very stable but had below average total carotenoid concentration. The check clones (91/02324, 30572, and TME 1) were also stable but with very low total carotenoid contents. Clones 01/1368, 01/1663, and 01/1371 had high total carotenoid concentration; but 01/1663 and 01/1368 were relatively unstable for this trait. Ubiaja (in 2006), Ibadan (in 2005 and 2006), and Zaria (in 2005) environments were the most stable for total carotenoid concentrations. Zaria (in 2006) was the most productive environment despite being relatively unstable. Mokwa (2005 and 2006), Ubiaja (2006), and Zaria (2006) environments had above average performance for total carotenoid concentration but Ubiaja (2006) was the most stable environment for this trait. Conversely, Mokwa (in 2005) was the most unstable environment although it had above average performance for total carotenoids.

The most stable clones for fresh yield with above

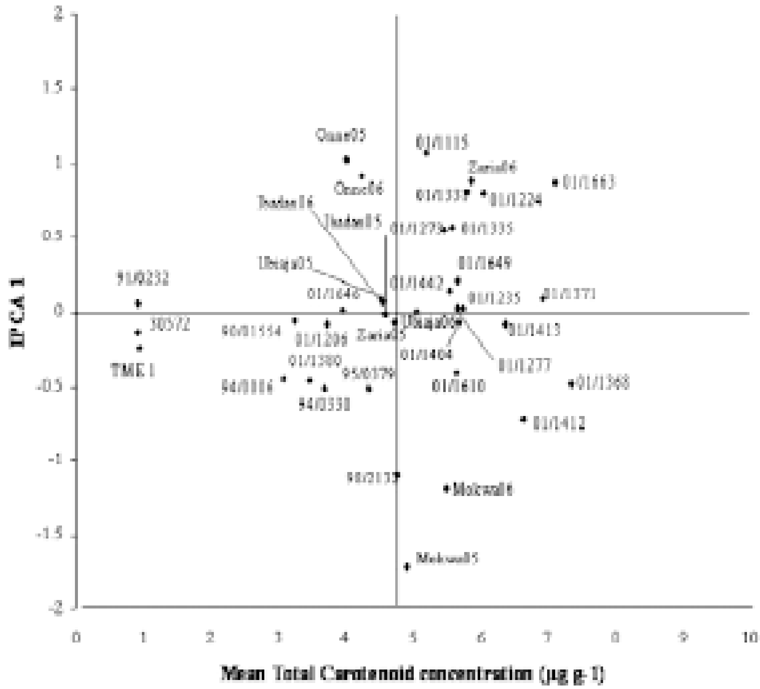

Figure 1: Biplot of mean total carotenoid concentration and the first Interaction Principal Components Axis (IPCA1) scores of 24 yellow fleshed and three white-fleshed cassava clones planted at five locations in Nigeria, 2004/05 and 2005/06.

average performance were 01/1235, 94/0006, and 01/1206 (Fig. 2); they performed best in the Ibadan environment of 2006. The check clone 91/02324 was high yielding and moderately stable. Clones 01/1331, 01/1115, 01/1277, and 01/1649 were stable but low yielding. Clone 98/2132 was the highest yielder but was also relatively unstable; it performed best in Ibadan (2006). Clone 01/1663 with about average yield was

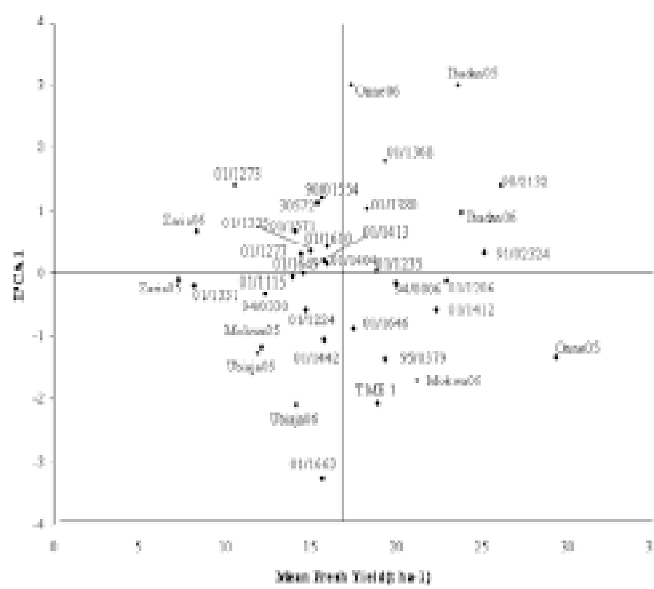

Figure 2: Biplot of mean fresh yield and the first Interaction Principal Components Axis (IPCA1) scores of 24 yellow fleshed and three white-fleshed cassava clones planted at six locations in Nigeria, 2004/05 and 2005/06.

Table 3. Analysis of variance (Matmodel) for total carotenoid concentration and fresh yield of 24 yellow and 3 white-fleshed cassava genotypes evaluated at five locations in Nigeria, 2004/05-2005/06.

Source	DF	SS	MS	Probability	% oftotal SS	% ofG x E SS
Total carotenoid concentration						
Total	533	3494.458	6.556			
TRT	269	3327.633	12.370	0.0000000 ***	95.2	
GEN	26	2576.173	99.084	0.0000000 ***	73.7	
ENV	9	230.782	25.642	0.0000000 ***	6.6	
G X E	234	520.678	2.225	0.0000000 ***	14.9	
IPCA 1	34	149.427	4.395	0.0000000 ***		28.7
IPCA 2	32	106.343	3.323	0.0000000 ***		20.4
IPCA 3	30	87.099	2.903	0.0000000 ***		16.7
IPCA 4	28	61.428	2.194	0.0000001 ***		11.8
IPCA 5	26	55.499	2.135	0.0000003 ***		10.7
IPCA 6	24	32.076	1.337	0.0023042 **		6.2
IPCA 7	22	12.756	0.580	0.5718793		2.4
Residual	38	16.049	0.422	0.9327101		3.1
Error	264	166.825	0.632			
Fresh yield						
Total	794	79642.37	100.305			
TRT	269	64423.13	239.491	.0000000 ***	80.9	
GEN	26	12952.11	498.158	.0000000 ***	16.3	
ENV	9	39566.95	4396.327	.0000000 ***	49.7	
G X E	234	11904.08	50.872	.0000001 ***	14.9	
IPCA 1	34	2986.42	87.836	.0000001 ***		25.1
IPCA 2	32	2958.239	92.445	.0000000 ***		24.9
IPCA 3	30	2061.578	68.719	.0000782 ***		17.3
IPCA 4	28	1378.005	49.214	.0150256 *		11.6
IPCA 5	26	1001.397	38.515	0.1297607		8.4
IPCA 6	24	498.408	20.767	0.8369402		4.2
IPCA 7	22	456.956	20.771	0.8244721		3.8
Residual	38	563.077	14.818	0.9937488		4.7
Error	525	15219.23	28.989			

Grand mean total carotene=4.7949; Grand mean fresh yield=16.9064 t/ha. ***, **, * Significant at P<=0.001, P<=0.01, and P<=0.05 respectively. $IPCA_i$ =Interaction principal component axis.

the most unstable across environments; it was, however, best adapted for Ubiaja (in 2006). Onne (2005) environment was the most productive for fresh yield and Zaria (in 2005) the most stable for yield although its mean performance was inferior. Ibadan and Onne were the most unstable environments for yield in 2005 and 2006 respectively, but had above average yields.

Discussion

There were variable responses to the impact of environment on the two traits of YF cassava. The environment effect was pronounced on both traits although the magnitude was higher for yield compared to total carotenoid contents. Partitioning of the sources of variation revealed that G x L was the main contributor of GEI for total carotenoid concentration and yield indicating that locations contributed more to fluctuations in performance than years. This suggests that breeding for both traits should involve testing varieties across multilocations to identify stable varieties. However, the high G and low E effects, and the relatively low GEI for total carotenoid concentration may necessitate evaluation over fewer environments to distinguish clones with high and stable performance. Studies on sweet potato (Grüneberg et al., 2005) also report G x E interactions of nutritional traits (that include carotenes) smaller than that of genetic variations. In this study, the GEI (AMMI) for total carotenoid concentration (14.9%) was close to the G effect on yield (16.9%). However, studies in late-maturing varieties of maize across locations (Oikeh et al., 2003) revealed no significant GEI effect on ^{2}-carotene.

The high G but low E effect on total carotenoid concentration confirms the qualitative nature of the trait. Qualitative traits are generally controlled by a few genes and are less prone to environmental effects. Gregorio (2002) reported that two genes possibly determine the inheritance of ß-carotene root-concentrations. Our studies on the inheritance of root flesh colour (data not published) which is related to carotenoid concentration also suggest the involvement of two genes. The high impact of environment on yield is expected, since yield is a polygenic trait (Easwari and Sheela, 1998; Cach et al., 2006) and, therefore, subject to much influence from the environment. The high environmental impact makes future potential genetic gain in the yield of YF cassava problematic. This may require early testing of clones in multi-environments to identify those with specific adaptations.

The relatively high stability of the clones for total carotenoid concentration suggests good prospects for cassava improvement for this trait. Clones with high carotenoid levels can be identified early in the breeding cycle and selection for the trait in a single environment can be relied upon. However, the significant GEI (even though of a small magnitude) on the trait suggests that some genotypes may not respond positively. Out of the YF clones, only 01/1235 was relatively stable with a high level of performance for both traits. This clone, therefore, can provide a genetic basis for breeding YF clones with high performance and stability for both yield and carotenoid concentrations. Overall, our results suggest that it is possible to breed YF cassava clones with high and stable performance for both yield and carotenoid concentrations.

Acknowledgements

This research was funded by HarvestPlus and the International Institute of Tropical Agriculture (IITA).

References

Cach, N.T., Lenis, J.I., Perez, J.C., Morante, N., Calle, F., and Ceballos, H. 2006. Inheritance of useful traits in cassava grown in sub-humid conditions. Plant Breed., 125: 177–182.

Chávez ,A.L., Sánchez, T., Jaramillo, G., Bedoya, J.M., Echeverry, J., Bolanos, E.A., Ceballos, H., and Iglesias, C.A. 2005. Variation of quality traits in cassava roots evaluated in landraces and improved clones. Euphytica, 143: 125–133.

Easwari, A.C.S. and Sheela, M.N. 1998. Genetic analysis in a diallel cross of inbred lines of cassava. Madras Agric. J., 85: 264–268.

Fernandez, G.C.J. 1991. Analysis of genotype x environment interaction by stability estimates. Hortic. Sci., 26: 947–950.

Gauch, H.G. and Furnas, R.E. 1991. Statistical analysis of yield trials with MATMODEL. Agron. J., 83: 916–920.

Gregorio, G.B. 2002. Progress in breeding for trace minerals in staple crops. J. Nutrition, 132: 500–502

Grüneberg, W.J., Manrique, K., Zhang, D., and Hermann, M. 2005. Genotype x environment interactions for a diverse set of sweet potato clones evaluated across varying ecogeographic conditions in Peru. Crop Sci., 45: 2160–2171

Iglesias, C., Jorge, M., Chávez, L., and Calle, F. 1997. Genetic potential and stability of carotene content in cassava roots. Euphytica, 94: 367–373.

Oikeh, S.O., Menkir, A., Maziya-Dixon, B., and Glahn, R.P. 2004. Micronutrient enhancement of maize in Nigeria, assessment of iron, zinc, and ^{2}-carotene content and iron bioavailability in elite maize varieties. IITA Monograph, IITA Ibadan, Nigeria, 39p.

Rodriguez-Amaya, D.B. and Kimura, M. 2004. *HarvestPlus Hand book for Carotenoid Analysis*. HarvestPlus Technical Monograph 2, International Food Policy Research Institute (IFPRI) Washington, DC USA, International Center for Tropical Agriculture (CIAT): Cali, Colombia 57p.