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Parent-offspring regression analysis for total carotenoids and some agronomic traits in cassava

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Abstract Six cassava cultivars, three yellow and three white-fleshed roots were crossed in a 3 × 3 topcross mating design to generate nine F_1 populations. One thousand, one hundred and ten botanical seeds from the 9 populations were sown in pots and maintained for 42 days in a screenhouse. The emerged seedlings were transplanted to the field in April, 2010 alongside their parents (from stem cuttings), family by family. Four hundred and sixty-four progenies survived and were harvested. Both field and laboratory data were used to evaluate total carotene content (TCC), dry matter content (DMC), storage fresh root yield (SFRY) and other root quality traits. Seed germination for different populations ranged between 15.5 and 80.9 % with a mean of 43.19 %. Phenotypic variation in DMC, TCC, SFRY, biomass, root number, harvest index and cassava mosaic disease (CMD) were recorded in all the families. Average values for the populations were TCC: 4.59 μg g^{-1}, DMC, 33.58 % and SFRY, 18.42 t ha^{-1}. Narrow sense heritability by midparent-offspring regression analysis and genetic gains were estimated for TCC, DMC, SFRY and reaction to CMD. TCC, DMC and CMD gave high heritability estimates of 0.73, 0.83 and 0.84, respectively. SFRY, on the other hand, had a low heritability estimate (0.15). TCC was negatively correlated with DMC across all evaluation stages and locations. There were very high levels of variation in the segregating F_1 progenies for all the traits. Also, narrow sense heritability estimate showed that genetic factors played a more important role than environmental factors for TCC, DMC and CMD, suggesting that reliable selection with simple recurrent phenotypic selection would be rewarding.

Keywords Carotenoids · Heritability · Midparent-offspring regression · Clone · Cassava

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Introduction

In cassava breeding, parental lines are usually selected based on their performance per se and little progress has been made using general combining ability as a criterion for parental selection (Ceballos et al. 2012).

Major challenges in cassava genetic improvement are the heterozygous nature of the progenitors used in

breeding, the low multiplication rate and long breeding cycle. Heterozygous parents are crossed to initiate a breeding population. The resulting F_1 seed are not uniform but segregate for traits of interest. Normally up to ten cuttings can be obtained from these plants resulting in a limited number of clones available from each new population. Therefore, the selection process usually takes about 6 years from the time the botanical seeds are germinated until enough planting materials are generated for multiplication in replicated trials. As a result, it can take 8–13 years to breed a new variety of cassava. However, efforts are being made to shorten this breeding cycle through selection of some traits such as pests and diseases resistance, yield, plant architecture, root quality traits such as dry matter content (DMC), functional properties of the starch, cyanogenic glycosides content, carotenoinds content, and cooking quality at early stages of selection (Ceballos et al. 2013).

Moderate to high heritability values have been reported for DMC in the tuberous roots (Mahungu 1987), showing that selection of genotypes at an early stage is possible. Kawano (1978) showed that selecting for high harvest index (HI) in early stages of the selection process (seedling plants nurseries and in single–row evaluations) is more effective in identifying high yielding genotypes than using root yield itself as a selection criterion. Root number, a trait affecting root yield, is known to be determined early in the growth cycle (Hunt et al. 1977), and can be used as an early selection criterion. However, storage root number can be affected by growing conditions (Hunt et al. 1977).

Cassava is one of the crops targeted for biofortification under Harvest-Plus Initiative as it is consumed daily by populations in Sub-Saharan Africa especially in Nigeria (Bouis et al. 2011). The tuberous root is composed of water (60–65 %); carbohydrate (30–35 %); fibre (0.8–1.3 %); ash (0.3–1.3 %), crude protein (0.03–0.60 %) and other extracts (0.2–0.6 %) (Rickard and Behn 1987). For the world food balance sheet in 2013, cassava ranked as the fifth staple crop in terms of kcal/capita/day behind rice, wheat, maize, and potato (FAOSTAT 2013). In Africa, cassava ranked the same as rice after maize and wheat.

Although it is a rich source of calories, cassava roots do not provide complete nutrition or a balanced diet. The tuberous root is low in vitamins, minerals, fats and protein and the roots contain cyanogenic glucosides that may reach toxic levels. Rapid techniques for screening cassava roots for minerals and carotenoids are currently been developed (Sánchez et al. 2014). There is extensive genetic variation among the wild relatives and landraces of cassava for carotene content (Chávez et al. 2005). They can be collected and evaluated and breeders can exploit additive gene effects, transgressive segregation, and heterosis to improve carotene density (Iglesias et al. 1997).

The potential for rapid increase in carotenoids content in cassava roots through cycles of recurrent selection is possible and proved to be highly efficient (Ceballos et al. 2013). Previous efforts had been able to increase the concentration from 0.42 μg g^{-1} of fresh roots in the base population to 1.34 μg g^{-1} after 2 cycles of selection and recombination (Jos et al. 1990). However, a high carotenoid content in the roots seems to be linked to a low DMC in African breeding populations. High DMC and fresh root yield are key traits for farmers to adopt new varieties. The objectives of this study were: (1) to evaluate the performance of full sib families for enhanced total carotenoids content (TCC), DMC, reaction to cassava mosaic disease (CMD) and storage fresh root yield (SFRY) in nine full sib families and (2) to estimate heritability for TCC, DMC, CMD and SFRY using parent-offspring regression analysis.

Materials and methods

Evaluation sites

The experiments were done at two sites in Nigeria. The site locations, climate and soil characteristics are indicated in Table 1. Rainfall is characteristically bimodal, with peaks in June and September and a period of less precipitation in August. The main dry season runs from December through February.

White and yellow root germplasm sources

The six progenitors used in this study were selected based on their characteristics of good performance in terms of pest and disease resistance, plant architecture, nutritional quality and flowering ability. Three of the parents (TMS 01-1368, TMS 05-0473 and TMS 05-1636) produced roots with yellow parenchyma (a

Table 1 Ecological factors and study conditions of locations used for clonal evaluation of the selected high carotene content genotypes

Ecological factors and study conditions	Umudike	Otobi
Latitude	05°29N	07°20N
Longitude	07°24E	080°41E
Altitude (above sea level)	120 m	319 m
Agro-ecology	Humid forest	Derived savanna
Annual rainfall (amount or range)	2289 mm	1500 mm
Temperature range (mean)	22–39 °C	23–41 °C
Relative humidity (range)	50–95 %	Not available
Soil classification	Dystric Luvisol	Ferric Luvisol

Sources NRCRI agrometerological unit

trait closely correlated with higher levels of carotenoids) and the other three (TMS 97-2205, TMS 98-0002 and TMS 98-0505) produce white-fleshed roots but had high DMC. Progenitors were planted in a crossing block at Western Farm of NRCRI in May, 2009. The six parents were arranged in a 3 × 3 topcross mating design (Table 2). A modified controlled hand pollination was carried out. Matured male flowers were collected shortly before opening against the recommendation by Kawano (1980) that male flowers should be picked after their anthesis. This option was adopted to avoid loss of the pollen as a result of forceful opening of the flowers and invasion by bees immediately after the flowers started opening (which would result in pollen contamination). Seeds were collected 2 months after pollination, sorted, labeled and stored. Percentage seed set was determined by dividing the number of fruits with the total number of seeds per cross.

Table 2 Nine cassava hybrid populations developed from six parents

Population	Female parent	Male parent
B1	TMS 98-0002	TMS 01-1368
B2	TMS 98-0002	TMS 01-1636
B3	TMS 98-0002	TMS 01-0473
B4	TMS 98-2205	TMS 01-1368
B5	TMS 98-2205	TMS 01-1636
B6	TMS 98-2205	TMS 01-0473
B7	TMS 98-0505	TMS 01-1368
B8	TMS 98-0505	TMS 01-1636
B9	TMS 98-0505	TMS 01-0473

All the female parents have white fleshed roots and every male parent produce yellow fleshed roots

Field evaluation and data collection

For each family the number of seedlings that germinated was recorded on 7, 14, 21 and 40 days after planting (DAP). There was no further emergence of seedlings 40 DAP. The percentage germination for each F_1 family was calculated as number of emerged seedlings divided by the number of seeds sown and multiplied by 100. A total of 473 seedlings across the nine F_1 families were vigorous enough to be transplanted at a spacing of 0.75 m × 1 m on April 30, 2010 at Umudike. Stem cuttings of the six parental varieties were also planted alongside the hybrid seedlings. Thirty cuttings of each parental variety were planted in 3 replicates at a spacing of 1 m × 1 m. Soil samples were collected by random sampling and analyzed for their physical and chemical properties. The field was weeded whenever necessary. The plants were watered manually for the first 38 days after planting or transplanting because of lack of rain.

Harvesting of the seedling evaluation trial (SET) was done at 12 months after planting (MAP). Only 464 genotypes out of the 473 were eventually harvested and analyzed. Since there is considerable within-family variation in full sib families of cassava (Ceballos et al. 2012), each plant was evaluated independently. The progenitors were also evaluated. For the clonal evaluation trials (CETs), both the F_1 and the six parental varieties were planted in the two different locations (Table 1) in 2011. The experimental design at each location was RCBD with three replicates using three cuttings per genotype in each replicate. However, for the CETs only 150 genotypes were evaluated (genotypes included in CETs were selected based on the capacity of the seedling plants to produce at least six cuttings) in 2012.

Data was collected on the following parameters: fresh shoot weight (FSW) (partitioned into leaves + petiole and stems), number of tuberous roots (NTR), stump weight (SW), storage fresh root weight (SFRW, DMC, and TCC. The data collected were used to estimate total biomass (TB) (kg plant^{-1}), harvest index (HI) and SFRY (t ha^{-1}). Biomass and HI were estimated on fresh weight basis.

The hybrids and the parents were also scored for CMD 1, 3 and 6 MAP; cassava bacterial blight (CBB), cassava anthracnose disease (CAD) severity and incidence using the symptom severity scale of 1–5 (Ogbe et al. 2003). Reaction to cassava green mite (CGM) was also rated at 6, 9 and 12 MAP. Descriptive statistics were calculated using Genstat Version 12 (Payne et al. 2009). For statistics and genetic analysis genotypes were considered fixed effects, whereas locations were considered as random effects.

Laboratory analysis

At harvest, three commercial size roots from each plant were selected, washed, peeled, chopped and mixed to obtain a single homogenous sample. The sample was then divided into two sub-samples, one was used for DMC quantification and another for extraction and quantification of pro-vitamin A carotenoids. Carotenoids quantification was by spectrophotometry. The protocol used followed the procedure described in the Harvest-Plus Handbook and in Rodriguez-Amaya and Kimura (2004). Measurements were made for the seedling evaluation trial (464 harvested genotypes) and 150 clones at clonal evaluation trial in two locations.

Heritability estimates and statistical analyses

Mid-parent-offspring regression was performed to estimate narrow-sense heritability (Falconer 1989; Lynch and Walsh 1998). SAS (9.2 version) and Excel spreadsheet software were used.

A two-tailed Pearson correlation analysis was done using Excel.Genetic gains (GG) were estimated using the formula described by Falconer and McKay, in 1996:

$$GG = ih^2\sqrt{vp}$$

where i = the selection differential in standard deviation units (the value of 'i' at 10 % selection intensity (1.76) was used in the calculations, h^2 = narrow-sense heritability of the trait; and $\sqrt{vp}$ = square root of the phenotypic variance.

Results

Percentage seed set and germination

The percentage seed set among the nine families varied considerably. The highest percentage seed set was recorded for the cross between TMS 98-0002 and TMS 05-0473 (75.34 % of potential seed set). The lowest percentage seed set (25.29 %) was observed in family B7 (TMS 98-0505 × TMS 01-1368).

Of the 959 seeds sown from the nine families, those from family B3 (TMS 98-0002 × TMS 05-0473) had the highest germination rate (80.9 %). This was followed by family B4 (TMS 97-2205 x TMS 01-1368) with 72.1 % seed germination. The lowest germination (15.5 %) was observed in family B9 (TMS 98-0505 x TMS 05-0473).

Seedling evaluation trial (SET)

TCC ranged from 0.5 to 15.71 μg g^{-1} with an average across the nine families of 3.3 μg g^{-1} (Table 3). Genotypes B1-51 and B1-19 (from the same cross: TMS 98-0002 × TMS 01-1368), had the highest TCC values with 15.71 and 15.24 μg g^{-1}, respectively. The lowest TCC values were observed in genotypes from family B9 (0.57 μg g^{-1}). DMC ranged from 9.19 to 49.08 % with a mean of 33.12 %. CMD average severity scores were 1.2, 1.6 and 2.09 for 1, 3 and 6 MAP respectively. Sixty-five percent of the genotypes were resistant (1–1.5), while 10 % were susceptible (Table 3).

Clonal evaluation trials

There were variations among the parents and their progeny in the two locations (Table 4). For instance, at Umudike parents TMS 01-1368 had the highest TCC (8.5 μg g$^{-1)}$ while parents TMS 98-0505 had the lowest TCC (1.2 μg g^{-1}). Parents TMS 98-0505 had

Table 3 Descriptive statistics of total carotenoids content (TCC), percentage dry matter (DMC), storage fresh root yield (SFRY) and cassava mosaic disease (CMD) at seedling evaluation stage in 2010 at Umudike

Family	Mother	Father	Size	Average	Minimum	Maximum	St. Dev.	Skewness	St. Error	Mother	Father	Size	Average	Mini-mum	Maxi-mum	St. Dev.	Skewness	St. Error
TCC										DMC								
B1	0.9	6.6	119	4.0	0.86	15.7	2.69	2.32	0.25	33.53	29.48	119	34.67	18.58	43.84	5.55	−0.8	0.51
B2	0.9	5.5	55	3.5	1.72	8.26	1.24	1.65	0.17	33.53	29.65	55	34.19	19.33	44.99	5.11	−0.93	0.69
B3	0.9	4.2	158	2.6	0.69	5.54	1.01	0.74	0.08	33.53	36.96	158	33.57	13.84	46.68	5.54	−0.92	0.44
B4	1.1	6.6	44	5.1	1.73	10.75	2.03	0.30	0.31	39.19	29.48	44	29.63	9.19	49.08	7.27	0.08	1.1
B5	1.1	5.5	44	3.4	1.89	6.72	1.10	0.91	0.17	39.19	29.65	44	27.36	11.6	39.2	7.22	−0.39	1.09
B6	1.1	4.2	18	2.6	0.93	6.36	1.19	1.77	0.28	39.19	36.96	18	30.55	22.93	35.98	3.51	−0.43	0.83
B7	1.1	6.6	3	1.8	0.95	2.99	1.05	1.09	0.61	37.19	29.48	3	38.79	34.73	45.62	5.95	0.67	3.43
B8	1.1	5.5	12	2. 5	0.94	3.69	0.90	0.07	0.26	37.19	29.65	12	33.2	26.14	43.35	5.01	0.6	1.45
B9	1.1	4.2	10	2.1	0.57	4.66	1.38	0.52	0.44	37.19	36.96	10	36.22	29.18	43.84	4.41	−0.18	1.39
SRFY										CMD								
B1	40	24	119	36.3	6.5	73.5	15.24	0.46	1.39	3	2	119	1.68	1	4	0.7	0.68	0.06
B2	40	5	55	37.25	7	68	14.43	0.04	1.95	3	1	55	1.58	1	4	0.74	1.11	0.1
B3	40	19	158	35.61	6	69.5	14.54	0.19	1.16	3	2	158	1.59	1	4	0.91	1.36	0.07
B4	34	24	44	34.92	6.5	73.5	14.40	0.69	2.17	2	2	44	1.91	1	3	0.74	0.14	0.11
B5	34	5	44	36.83	6.5	67	14.63	0.03	2.21	2	1	44	1.46	1	3	0.63	1.03	0.09
B6	34	19	18	36.81	19	67	13.72	0.50	3.23	2	2	18	1.56	1	3	0.71	0.84	0.17
B7	19	24	3	41.33	36.5	62.5	13.53	0.53	7.81	1	2	3	1.67	1	2	0.58	−0.71	0.33
B8	19	5	12	30.04	10.5	66	15.67	0.94	4.53	1	1	12	1.25	1	2	0.45	1.16	0.13
B9	19	19	10	38.1	21.5	56.5	11.12	0.22	3.52	1	2	10	1.7	1	3	0.82	0.58	0.26

B stands for Family

Table 4 Mean of total carotene content, dry matter content, cassava mosaic disease and storage fresh root yield in the parents and progeny clonal evaluation trials at Otobi and Umudike location in 2011

Population	Otobi				Umudike			
	TCC	DMC	CMD	SFRY	TCC	DMC	CMD	SFRY
Parents								
TMS 01-1368	7.9	26.9	3.0	21.2	8.5	29.7	1.7	14.9
TMS 05-0473	5.1	35.8	2.7	17.9	4.0	26.6	1.7	9.6
TMS 05-1636	7.0	30.6	2.3	23.3	6.2	30.0	1.3	6.2
TMS 97-2205	1.8	33.4	2.0	37.7	2.3	32.7	2.0	27.2
TMS 98-0002	1.1	35.7	1.7	48.3	2.0	39.8	2.0	27.2
TMS 98-0505	1.4	38.8	1.6	52.4	1.2	39.9	1.0	35.1
Progeny								
B1	5.4	33.0	2.1	20.9	4.9	35.4	2.0	14.7
B2	5.1	33.6	2.1	24.8	3.4	37.3	1.9	16.5
B3	3.7	33.5	2.2	26.0	2.8	36.9	2.2	15.1
B4	8.7	27.8	2.3	16.6	5.6	30.9	2.2	12.1
B5	3.7	32.1	2.0	17.6	3.7	30.6	1.9	11.9
B6	4.9	29.0	1.8	23.4	3.7	31.7	1.6	14.2
B7	1.9	39.3	2.0	23.3	2.9	40.5	1.3	19.7
B8	3.6	32.1	2.0	14.6	4.1	31.9	1.6	11.2
B9	3.2	31.3	2.2	37.11	2.5	33.2	1.7	16.5

the highest DMC and SFRY of 39.9 and 35.1 % respectively. Also at Otobi location, parents TMS 01-1368 had the highest TCC (7.9 μg $g^{-1)}$ while parent TMS 98-0002 had the lowest TCC 1.1 μg g^{-1}. Also, parents TMS 98-0505had the highest DMC and SFRY (38.8 and 52.4 %) respectively. On the other hand, the progeny of cross B4 had the highest average TCC (5.7 μg g^{-1}), while those of cross B9 had the lowest TCC (2.5 μg g^{-1}) at Umudike. Also, at Otobi location, progeny of cross B4 had the highest average TCC (8.7 μg g^{-1}), while those of cross B7 had the lowest (1.9 μg g^{-1}). Also progeny of crosses B7 and B9 had the highest DMC and SFRY of 39.3 and 37.1 % respectively (Table 4).

Combined analysis of variance for CET at the two locations

A combined analysis of variance for TCC and other traits studied is presented in Table 5. The mean squares were highly significant ($P < 0.001$) for the model and crosses at the two locations for all the traits. Environmental effect mean squares were highly significant for all the traits studied. Also, highly significant variation between genotypes was seen for all the traits. Genotypes by environment interaction mean squares were significant for DMC, CMD and TCC, but not for SFRY. This implies that genotype effect is stable across the environment in some traits studied.

The coefficient of variation (CV %) was high for SFRY (68.1 %) and TCC (41.8 %) across both locations but low for DMC (10. 8 %), HI (27.8 %) and RTCOL (22.2 %) respectively. The R^2 values explained from 47 (RTNO) to 81 (DMC) % of the variations in the genotypes across the two locations (Table 5).

Selection parameters for advancement of genotypes

Selection of the genotypes that combined key traits (TCC, DMC, SFRY and CMD) across locations is shown in Table 5. Four clones, B1-29, B 1-35, B1-95 and B 4-37, were selected across the two locations based on the four traits and the selection index. They were selected in the following order: TCC $\geq$ 9.0; DMC $\geq$ 32.0, SFRY $\geq$ 20.0 and CMD $\leq$ 1.5 (Table 6). These genotypes would be advanced to preliminary evaluation stage.

Table 5 Combined analysis of variance for measured traits at Umudike and Otobi in 2011

SV	DF	CMD	RTNO	BIOMAS	HI	SFRY	DMC	RTCOL	TCC
Rep (Env)	4	3.63***	667.29***	611.59***	0.19***	1731.16***	21.99 ns	6.31***	5.04 ns
Env	1	5.79**	504.30**	1215.35***	2.46***	10,730.42***	857.41***	7.50**	130.10***
Gen	124	1.27***	104.57***	128.72***	0.02***	340.56***	175.65***	5.81***	23.17***
Gen*Env	124	0.83**	53.74 ns	63.51 ns	0.01 ns	143.78 ns	37.72***	1.51***	6.45***
Error	496	0.56	51.22	62.01	0.01	157.36	13.16	0.72	3.68
CV (%)		36.83	50.06	60.62	27.81	68.10	10.81	22.22	41.76
R^2		0.51	0.47	0.56	0.52	0.49	0.81	0.72	0.68
LSD (0.05)		1.97	8.12	8.93	0.14	14.23	4.12	0.96	2.18

SV Sources of variation, *DF* degree of freedom, *CMD* cassava mosaic disease, *RTNO* total root number, *HI* harvest index, *SFRY* storage fresh root yield, *DMC* dry matter content, *RTCOL* root colour, *TCC* total carotene content

ns, *, **, and *** denote non-significant at $P < 0.05$; significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$ respectively

Table 6 Averages of the nine families across locations and clones selected based on selection index- including TCC, DMC and SFRY

Clone	TCC (μg g^{-1})	DMC (%)	SFRY (t/ha)	CMD
B1	5.2	34.2	17.9	2.1
B2	4.2	35.4	20.7	2.0
B3	3.2	35.2	20.5	2.2
B4	7.2	29.4	14.3	2.2
B5	3.7	31.4	14.7	1.9
B6	4.3	30.4	18.8	1.7
B7	2.4	37.9	21.5	1.7
B8	3.9	32.1	12.9	1.8
B9	2.9	32.3	26.8	1.9
LSD (0.05)	2.18	4.12	14.23	1.97
B1-29	9.4	35.0	22.3	1.4
B1-35	9.0	36.8	22.1	1.2
B1-95	10.2	31.7	31.3	1.3
B4-37	9.2	34.9	20.6	1.2

B stands for clones from each family

Cassava pests and diseases

Both seedling and two clonal evaluation trials were evaluated for CMD, CBB, CAD and CGM for 3 consecutive periods: CMD (1, 3 and 6), CBB (3, 6 and 9), CAD (3, 6 and 9) and CGM (6, 9 and 12) months after planting (MAP) respectively. Combined ratings for mean severity of CMD at 1, 3 and 6 MAP was 1.15, 1.62 and 2.09; CBB at 3, 6, and 9 MAP (1.05, 1.47, 1.79), CAD at 3, 6 and 9 MAP (1.04, 1.65, 2.00) and CGM at 6, 9 and 12 MAP were 1.29, 1.21 and 1.62 respectively. About 65 % of the genotypes were resistant (1–1.5), 25 % were moderately resistant (1.6–2.4), 7 % were moderately susceptible (2.5–3.5) and 3 % susceptible (4.0–5.0) to CMD. Similar results were obtained for CBB, CAD and CGM across the 9 families at the two locations. The result also showed that the 6 parents' cultivars are resistant (1.0–1.5) but their offspring (genotypes) were variable in reaction to the pest and diseases scored. The four genotypes selected for advancement are resistant to CMD, CBB, CAD and CGM respectively.

Correlation

Highly significant but negative correlation was observed between TCC and DMC in the two locations (Table 7). This would suggest that the deeper the flesh colour (from white to orange) and TCC values, the lower the dry matter content of the root. Also, there

Table 7 Correlation coefficients between total carotene content and other agronomic traits evaluated at CET at Otobi and Umudike in March, 2012

	CMD	DM	SFRY	TCC
Otobi				
CMD	1			
DM (%)	−0.097^{ns}	1		
SFRY	−0.176**	0.110**	1	
TCC	0.012^{ns}	−0.224***	−0.152**	1
Umudike				
CMD	1			
DM	−0.064^{ns}	1		
SFRY	−0.113*	0.208***	1	
TCC	0.063^{ns}	−0.292***	−0.140**	1

Trait abbreviations *CMD* Cassava mosaic disease, *DMC* dry matter content, storage fresh root yield, *TCC* total carotene content, *CET* clonal evaluation trial

*, **, *** significant at $P < 0.05$, $P < 0.01$, $P < 0.001$; *ns* not significant at $P < 0.05$

was significant but negative (as expected) correlation between SFRY and CMD in both locations. There was significant and positive association between SFRY and DMC in both locations.

Heritability

Midparent-offspring regression estimates for percentage total carotenoids content, dry matter content, storage fresh root yield and cassava mosaic disease reaction are presented in Table 8. TCC had high heritability across the two locations and the combined analysis (0.73, 0.69 and 0.72, respectively). DMC had high heritability estimate at Umudike trial (0.82) but moderate heritability estimate at Otobi (0.46). Combined across locations the heritability for DMC was high (0.79). CMD severity showed high heritability across the two locations. SFRY on the other hand, had low heritability across locations.

Table 8 Estimates of heritability for total carotenoids content, dry matter content, storage fresh root yield and cassava mosaic disease at two locations and combined analysis

Traits	Heritability estimates		
	Umudike	Otobi	Combined
TCC (μg g^{-1})	0.73 ± 0.16	0.69 ± 0.49	0.72 ± 0.14
DMC (%)	0.82 ± 0.30	0.46 ± 0.29	0.79 ± 0.03
SFRY(t/ha)	0.30 ± 0.11	0.11 ± 0.13	0.15 ± 0.04
CMD	0.84 ± 0.18	0.76 ± 0.12	0.81 ± 0.18

Discussion

The number of seeds harvested differed per female showing that seed production may probably be genotype dependent; therefore, selection of highly fertile genotypes as female parents may be a critical factor in certain cases. Most of the harvested fruits in this study had three seeds at harvest, an indication that the female parents are highly fertile and productive. However, less than one seed was obtained per pollination on an average in this study. The observed number of female flowers pollinated and number of seeds harvested in each population indicates that success of pollination in cassava cannot be accurately predicted from the number of flowers pollinated.

Some of the genotypes were discarded at the early stage of the evaluation process due to low vigour which resulted in unavailability of enough planting materials for advancement and further evaluation of such genotypes at the clonal stage. Although enhanced carotenoids content in cassava was the aim of the study, low vigour genotypes will not make a good variety since cassava is vegetatively propagated. Discarding such genotypes at early stage of a breeding programme will help to concentrate better on a lower number of promising genotypes.

The estimates of correlation among traits are useful for planning a breeding program that is aimed at synthesizing a genotype with desirable traits. A negative correlation was observed between TCC and DMC in this study indicating that the deeper the flesh colour (high TCC) the lower the dry matter. This is common to most Africa and Indian yellow fleshed cassava germplasm (Vimala et al. 2008; Akinwale et al. 2010; Esuma et al. 2012). This challenge can be solved through screening of available accessions with yellow fleshed roots in Africa, and possibly, by introduction of improved high-carotenoids content germplasm from South America. It has been demonstrated that the initial negative linkage between TCC and DMC can easily be broken and that high carotenoids and DMC can be combined in a single genotype with appreciable yield (Ceballos et al. 2013).

In this study, we selected four genotypes with high TCC genotypes and adequate levels of DMC. This further indicates that the linkage between high TCC and low DMC can be broken.

Also, the phenotypic correlation between total biomass and fresh root yield in cassava has been observed to be very high (0.97) at the early evaluation stages, and lower (0.54) at an advanced stage of evaluation (Kawano et al. 1998). Kawano et al. (1998) reported a very low correlation (−0.19) between HI and fresh root yield at the early stages (seedling and clonal), but very high (0.93) at advanced evaluation stages. Cassava genotypes perform differently in terms of yield, harvest index and biomass at different stages of evaluation and selection (Kawano and Thung 1982). Those genotypes with higher biomass at seedling or clonal evaluation trials tend to dominate others with less biomass in competition for light. Similarly, genotypes with high harvest index may be weak competitors while those with greater biomass tend to be strong competitors. The positive correlation observed in this study implies that the higher the SFRY, the higher the DMC. It may probably indicate that both traits can be selected during early stage of breeding program.

Genotype by environment interaction was significant for CMD, DMC, RTCOL and TCC. This is in agreement with Maroya et al. (2012) who found highly significant difference among 21 yellow cassava varieties for TCC, DMC, CMD and other traits evaluated in five cassava growing states for 2 years in Nigeria. Measurement of GxE interaction is important for the determination of an optimum breeding strategy for releasing genotypes with adequate adaptation to target environments (Baiyeri et al. 2008). Most genotypes performed well for both TCC and DMC in Umudike location but were low or intermediate in Otobi location. This emphasis the need to select the genotypes for each location based on the data obtained from that location.

Midparent-offspring regression analysis estimated heritability for TCC, DMC, SFRY and CMD. This is a good estimator of narrow sense heritability (Hallauer and Miranda 1988; Lynch and Walsh 1998). The narrow sense heritability for TCC, CMD and DMC were found to be high (except for DMC at Otobi). This indicated that the additive nature of genetic variation would be transmitted from parents to the offspring and, therefore, significant genetic progress can be made.

Conclusion

Over 50 % of the progenies had higher TCC than their parents, and acceptable levels of DMC and SFRY. Four clones (B1-19, B1-51, B1-24 and B4-25) were selected across locations based on, TCC, DMC, CMD and SFRY. Clone B4-37 has the best combination of TCC, DMC and SFRY (8.2, 34.9 and 20.6) respectively.

Consequently, the results of these studies suggest that most of the yellow cassava clones used hold promise for resistance to the CMD, CBB, CAD diseases and CGM pest, and could, therefore, be investigated further to obtain the most suitable clones for the cultivation of the relatively more nutritious yellow cassava in Nigeria.

Significant genetic diversity was generated from these crosses and can be utilized to develop carotenoids-rich cassava varieties and upgrade existing ones. Apart from combating vitamin A deficiency among cassava consuming Nigerians, carotenoids-rich cassava varieties can also be used to supplement yellow maize in animal feed, particularly egg production in poultry.

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