

Research Paper

Diallel analysis of provitamin A carotenoid and dry matter content in cassava (*Manihot esculenta* Crantz)

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Global efforts are underway to biofortify cassava (*Manihot esculenta* Crantz) with provitamin A carotenoids to help combat dietary vitamin A deficiency afflicting the health of more than 500 million resource-poor people in Sub-Saharan Africa. To further the biofortification initiative in Uganda, a 6×6 diallel analysis was conducted to estimate combining ability of six provitamin A clones and gene actions controlling total carotenoid content (TCC), dry matter content (DMC) in cassava roots and other relevant traits. Fifteen F₁ families generated from the diallel crosses were evaluated in two environments using a randomized complete block design. General combining ability (GCA) effects were significant for TCC and DMC, suggesting the relative importance of additive gene effects in controlling these traits in cassava. On the other hand, non-additive effects were predominant for root and shoot weight. MH02-073HS, with the highest level of TCC, was the best general combiner for TCC while NASE 3, a popular white-fleshed variety grown by farmers in Uganda, was the best general combiner for DMC. Such progenitors with superior GCA effects could form the genetic source for future programs targeting cassava breeding for TCC and DMC. A negative correlation was observed between TCC and DMC, which will require breeding strategies to combine both traits for increased adoption of provitamin A cassava varieties.

Key Words: biofortification, cassava, combining ability, genetic improvement, provitamin A carotenoid.

Introduction

Cassava, the fifth most important staple crop in the world, is a widely grown and consumed root crop in Sub-Saharan Africa (SSA) (Tan 2015). The crop is central to food and income security throughout SSA, especially among resource-poor farmers (Fermont *et al.* 2010). The popularity of cassava is attributed to its ability to produce reasonable yields under poor soil conditions and extended droughts, where other food crops would practically fail (El-Sharkawy 2007). In Uganda, the area harvested under cassava has steadily increased from 405,000 ha in 2003 to 440,000 ha in 2013 (FAOSTAT 2014), despite the known enormous threat from cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) during this period (Alicai *et al.* 2007). This trend in cultivation of cassava is a reflection of the growing relevance of the crop in people's livelihoods.

However, compared to efforts in other important food crops, genetic improvement of cassava has lagged behind,

keeping the crop's production below its potential (Ceballos *et al.* 2015). For example, average fresh root yield of cassava reported for Uganda is 12 t ha⁻¹, which is lower than that for Ghana (18.3 t ha⁻¹), Thailand (21.8 t ha⁻¹) and the Caribbean (22.1 t ha⁻¹) (FAOSTAT 2014). In Africa, formal cassava breeding began in the 1930s at Amani research station, Tanzania, when scientists Storey and Nichols guided the first systematic efforts to breed for CMD and CBSD resistance (Nichols 1947, Storey and Nichols 1938). Subsequent cassava breeding activities only intensified after the colonial rule when an organized program was instituted at International Institute for Tropical Agriculture (IITA), Ibadan, in the 1970s (Umanah 1977). Although efforts are now reshaping breeding strategies to exploit the yield potential of cassava, they lag considerably behind what has been achieved in, for example, maize since the 1900s after a breakthrough was recorded in use of inbred lines to exploit heterosis for increasing grain yield (Shull 1908).

Most agronomic traits including yield, dry matter content (DMC) and certain types of disease resistance are quantitatively inherited in cassava, and information about their mode of inheritance helps breeders to use methods that increase genetic gain (Calle *et al.* 2005, Kulembeka *et al.* 2012). During early stages of cassava breeding, open pollination

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schemes were the most predominant mating designs used to generate breeding populations upon which selection could be imposed (Kawano 2003). These designs involved establishing selected progenitors of complementary traits *per se* in crossing nurseries to generate half-sib progenies. Although these mating schemes are simple to implement, they are limited in that they do not provide for estimation of specific combining ability (SCA), which is important in inheritance of key traits like fresh root yield (Ceballos *et al.* 2004, 2015, Crossa *et al.* 2010). Subsequent shifts in cassava breeding schemes have seen an increased production of full-sib progeny (Ceballos *et al.* 2012, Nassar and Ortiz 2006). The full-sib crossing schemes employ controlled pollinations, where selected mating designs are used to generate families from specific parental combinations, facilitating genetic studies alongside production of breeding populations (Nduwumuremyi *et al.* 2013).

The diallel mating design, specifically, has become popular for cassava breeding because it facilitates generation of useful information on genetics of key agronomic traits and allows identification of parents with superior combining ability for developing breeding populations (Kulembeka *et al.* 2012, Tumuhimbise *et al.* 2014, Zacarias and Labuschagne 2010). It is this genetic information that guides breeders to deploy appropriate methods for crop improvement (Acquaah 2012, Nduwumuremyi *et al.* 2013). Knowledge of general combining ability (GCA) of parental lines is particularly helpful for predicting genetic gains in a breeding program (Falconer and Mackay 1996). Hayman (1954) and Griffing (1956) elaborated on the procedure for statistical analyses based on diallel data, which partitions total variation into GCA of the parents and SCA of crosses.

To leverage vitamin A deficiency (VAD) afflicting more than 500 million people in SSA alone, a novel effort referred to as HarvestPlus Challenge Program was initiated to biofortify staple crops (Mayer *et al.* 2008). Subsequent efforts to develop cassava varieties enriched with provitamin A carotenoids (pVAC) was initiated, under a joint stewardship of International Center for Tropical Agriculture (CIAT) and IITA. On this premise, the national cassava breeding program of Uganda initiated a breeding program with the objective of developing high yielding varieties with high levels of pVAC (Esuma *et al.* 2012). Deployment of such carotene-rich cassava would sustainably improve nutrition and reduce prevalence of VAD in communities that primarily depend on cassava (Mayer *et al.* 2008, Nassar and Ortiz 2010), particularly so in Uganda where the crop is the second most consumed after bananas. Ideally, pVAC cassava varieties are also expected to have high DMC. Varieties that combine these two traits are more likely to be adopted for subsistence agriculture (Njukwe *et al.* 2013). The strong negative correlation that has been reported between DMC and total carotenoids content (TCC) in the African cassava germplasm (Akinwale *et al.* 2010, Njoku *et al.* 2015) could present a potential challenge for cassava breeding programs tasked to improve both traits. Other studies analyzing Latin

American cassava germplasm suggested that correlations between carotenoid content and DMC are not high enough to reach statistical significance (Chávez *et al.* 2005, Sánchez *et al.* 2014). Against this background, the current study was conducted with specific objectives to (1) estimate the combining ability of six cassava progenitors for TCC and DMC and (2) determine gene actions controlling these two traits in cassava roots.

Materials and Methods

Experimental sites

The crossing block for the study was established in November 2011 at the National Crops Resources Research Institute (NaCRRI) located within the Lake Victoria crescent at 32°37'36.0"E and 0°31'13.7"N, 1,134 m above sea level. The seedling trial was established at Abi Zonal Agricultural Research and Development Institute (Abi-ZARDI) located at 31°1'28.4"E and 2°36'33.3"N, 1,060 m above sea level, in a region with low CBSD pressure. This consideration was important to ensure that the planting materials for clonal trials were free of CBSD to minimize the necrotic root symptoms of the disease (Hillocks and Jennings 2003) for analysis at clonal stage. Clonal trials were conducted at NaCRRI and Abi-ZARDI during 2014–2015. Both locations experience a bimodal rainfall pattern, with two rainy and dry seasons of almost equal length. The first season of rainfall peaks between March and mid-June and second season rains peak from August to November. Temperature and rainfall data were recorded during the experimentation period as well as the soil nutrient profile of the fields before planting the trials, and these varied within the range reported to be ideal for cassava production (Cadavid 2012, Hauser *et al.* 2014) (**Table 1**).

Progenitors and hybridization

Six genetically diverse clones in advanced selection stages were used as progenitors (**Table 2**). The six progenitors were planted in the crossing block at Namulonge under rain-fed conditions in paired rows to facilitate generation of

Table 1. Description of weather and soil conditions at experimental sites for clonal evaluation

Parameter	Unit of measure	Critical value	Abi-ZARDI ^d	Namulonge
pH		4.0–8.0*	6.1	6.0
OM ^a	%	3.0	6.6	7.8
N	%	0.2	0.3	0.4
P	ppm	10.0*	3.0	2.4
Ca	ppm	50.0*	1,141.6	1,248.9
Mg	ppm	14.3*	336.2	734.2
K	ppm	58.5*	336.2	443.2
Rainfall	mm		884	1264
T (min) ^b	°C		15.9	17.4
T (max) ^c	°C		31.4	29.8

^a Organic matter; ^b Minimum temperature; ^c Maximum temperature;

^d Abi Zonal Agricultural Research and Development Institute; Values with asterisks are critical for cassava (Cadavid 2012).

Table 2. List of progenitors used in the 6×6 diallel study

Genotype	Code	Source	RFC ^a	Salient traits
NASE 3	P1	IITA ^b	White	High DMC ^c ; CMD ^d and CBSD ^e tolerance
CPCR24B-10	P2	CIAT ^f	Light yellow	pVAC ^g , CMD resistance
MH05-2870	P3	IITA	Yellow	pVAC, CMD resistance
MH05-0233	P4	IITA	Yellow	pVAC, CMD resistance
CPCR15B-26	P5	CIAT	Yellow	pVAC, CMD resistance
MH02-073HS	P6	IITA	Deep yellow	pVAC, CMD resistance

^a Root flesh color; ^b International Institute for Tropical Agriculture;

^c Dry matter content of roots; ^d Cassava mosaic disease; ^e Cassava brown streak disease; ^f International Center for Tropical Agriculture;

^g Provitamin A carotenoids.

the 15 F₁ families of a 6×6 diallel design. Planting was done at a spacing of 1.5 m between pairs of progenitors for crossing and 2 m alleys between subsequent progenitor pairs, which collectively provided the additional space to ease movement during the pollination process. Controlled pollinations were performed by hand following the standard procedures described by Kawano (1980), with special care given to cover mature flowers with nylon-meshed pollination bags 2–3 days before and after pollination. Botanical seeds were harvested from mature fruits within 2.5–3 months after pollination and stored in paper bags for two months to break dormancy. At least 100 seeds from each of the 15 F₁ families were germinated in plastic pots filled with natural forest soil, in a screen house. After germination, seedlings were watered routinely whenever deemed necessary to ensure vigorous growth.

Field evaluation

Seedlings from the 15 F₁ families were grown in the field during 2013–2014 for the purpose of generating planting materials for replicated clonal trials. At 12 months after planting (MAP), 20 genotypes with the ability to generate ≥12 standard-size cuttings (i.e. 4–6 nodes) were randomly selected from each F₁ family to constitute the population for clonal evaluation. At each site, trials were laid out in a randomized complete block design with two replications. A single row plot of three plants represented each entry, with 2 m alleys between blocks. Planting was done at a spacing of 1 × 1 m, giving a total population of 10,000 plants ha⁻¹. These clonal trials were planted in May 2014 and harvested for root measurements in April 2015. Progenitors were evaluated alongside the progeny in the clonal trials, following similar planting pattern. Weeding was done as necessary and trials rain-fed.

Measurement of root yield, dry matter content and total carotenoid content

During harvesting, all plants in a plot were uprooted and the biomass bulked to estimate yield components by separately weighing the fresh roots (kg plant⁻¹) and foliage (kg plant⁻¹) using a Salter Brecknell suspended weighing scale [model: 23510S(SHFSB-0404)] calibrated in kilograms.

Harvest index (HI) was computed from the measure of fresh root weight (FRW) and fresh shoot weight (FSW) using the formula:

$$HI = \frac{FRW}{(FRW + FSW)}$$

Three roots (of an average size) were randomly selected from each plot, labelled and processed for measurement of DMC and TCC. These roots were peeled, washed under running water and dried with a paper towel. The dried roots were cut longitudinally into four quarters. The opposite quarters of each of the three roots were pooled, chopped into small pieces and mixed manually to get a homogeneous sample. Approximately 200 g of each homogenous sample were taken for measurement of DMC by drying the sample in an oven to constant weight at a temperature of 105°C for 24 hours. The dried samples were reweighed to obtain their DMC, calculated as:

$$DMC (\%) = \frac{DSW}{FSW} \times 100$$

where DSW = dry sample weight and FSW = fresh sample weight.

The iCheck analytical kit developed by BioAnalyt Laboratory (<http://www.bioanalyt.com>) was used for measuring TCC (Ceballos and Parkes 2014). Briefly, 5 g of the homogenous root sample was pounded and ground into a smooth and fine paste using a mortar and pestle. To aid grinding of the sample, 20 ml of distilled water was added gradually and the resulting solution transferred into a 50 ml calibrated falcon tube. The falcon tube content was shaken thoroughly and 0.4 ml of the solution injected into the iEx™ CAROTENE vial using the syringe and needle provided with the kit. Vials were placed on a solid surface for approximately 5 min, shaken again and allowed to stand until two solution phases appeared inside the vial: a clear upper phase and a turbid lower phase. At this point, the absorbance of the vial content (the upper solution phase) was measured using the iCheck™ CAROTENE device. TCC was calculated as:

$$TCC (\mu\text{g g}^{-1}) = \frac{V_s}{W_s} \times A$$

where V_s = volume of solution transferred to the falcon tube, W_s = weight of a sample and A = absorbance of the iEx™ CAROTENE vial content at a wavelength of 450 nm. Each sample was extracted and measured for TCC once. All procedures for carotenoid quantification were performed in a dark room. All harvested root samples were analyzed for both TCC and DMC within 12 hours. Root flesh color was also scored for all genotypes using visual inspection following the standard color scale developed by CIAT.

Statistical analysis

Analysis of variance (ANOVA) was done using the Plant Breeding Tools software (PBTools) (PBTools 2014). Diallel analysis was conducted according to the Griffing (1956)

method 2, model I for fixed effects, to estimate the GCA and SCA effects. Briefly, method 2 is a diallel, where parents and one set of F_1 s, but not reciprocals, are included in the statistical analyses. Model I considers parents as fixed effects and interpretations of the genetic effects are limited to the specific set of parents used. Thus, GCA and SCA effects were estimated as:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$$

where: Y_{ijk} = observed value for the ij^{th} cross grown in the k^{th} replication/environment combination, μ = overall mean, g_i = GCA effect for the i^{th} parent, g_j = GCA effect for the j^{th} parent, s_{ij} = SCA of the cross between the i^{th} and j^{th} parents and e_{ijk} = error term associated with the ij^{th} cross evaluated in the k^{th} replication/environment. The relative importance of GCA and SCA effects for each trait was determined from the proportions of a family's sum of squares (SS) due to GCA and SCA. Further statistical inference was made using Baker's ratio (BR) calculated from estimates of the genetic parameters as:

$$BR = \frac{2MS_{\text{GCA}}}{(2MS_{\text{GCA}} + MS_{\text{SCA}})}$$

where MS_{GCA} and MS_{SCA} are mean squares due to GCA and SCA effects, respectively (Baker 1978). Pearson's correlation coefficients for phenotypes from the combined data across environments and replications were computed using the following method: $r_p = cov_{xy}/\sigma_x\sigma_y$, where r_p is the phenotypic correlation, cov_{xy} is phenotypic covariance between traits x and y while σ_x and σ_y are the standard deviations of the phenotypes x and y respectively. Genetic correlation coefficients among traits were calculated from the GCA effects as described by Hohls and Clarke (1995).

Results

Mean performance of the 15 F_1 families and their progenitors

For all genotypes evaluated across the 15 F_1 families, TCC values varied from 0 to $11.0 \mu\text{g g}^{-1}$, with the highest mean ($5.8 \mu\text{g g}^{-1}$) recorded for family $P5 \times P6$ and the lowest ($2.1 \mu\text{g g}^{-1}$) for family $P1 \times P2$ (Table 3). Individual DMC values for the evaluated genotypes ranged from 15.9 to 45.9%. At family level, DMC ranged from 22.2% for family $P5 \times P6$ to 34.4% for $P1 \times P2$. Family $P5 \times P6$ recorded the highest mean FRW ($2.4 \text{ kg plant}^{-1}$), while family $P2 \times P3$ recorded the lowest mean FRW ($1.2 \text{ kg plant}^{-1}$). The highest mean value of FSW ($5.5 \text{ kg plant}^{-1}$) was recorded for family $P1 \times P5$ and the lowest value ($2.4 \text{ kg plant}^{-1}$) for $P2 \times P4$. Meanwhile, family $P2 \times P3$ had the lowest mean value (0.28) for HI and family $P5 \times P6$ had the highest mean value (0.74) for HI. At progenitor level, genotype $MH02-073HS$ recorded the highest level of both TCC ($10.4 \mu\text{g g}^{-1}$) and FRW ($6.3 \text{ kg plant}^{-1}$), but had the lowest DMC (22.2%). NASE 3 recorded the highest DMC (37.3), but had very low TCC ($0.2 \mu\text{g g}^{-1}$). Fig. 1 illustrates phenotypic distribution of

Table 3. Performance of progenitors and their respective F_1 progeny across two locations in Uganda during 2014–2015

Parent/Family	Number ^a	TCC ^b	DMC ^c	FRW ^d	FSW ^e	HI ^f
NASE 3 (P1)	—	0.2	37.3	2.5	2.6	0.51
CPCR24B-10 (P2)	—	4.4	32.3	2.0	2.9	0.44
MH05-2870 (P3)	—	4.3	33.4	1.5	5.7	0.32
MH05-0233 (P4)	—	4.9	29.0	2.4	1.7	0.58
CPCR15B-26 (P5)	—	5.3	30.7	2.3	2.1	0.49
MH02-073HS (P6)	—	10.4	22.2	6.3	2.5	0.72
$P1 \times P2$	17	2.1	34.4	1.5	4.1	0.33
$P1 \times P3$	18	3.1	31.0	1.8	4.0	0.42
$P1 \times P4$	19	3.1	31.3	1.9	3.7	0.45
$P1 \times P5$	20	2.4	33.9	1.9	5.5	0.34
$P1 \times P6$	16	2.6	28.1	1.9	5.3	0.56
$P2 \times P3$	15	2.7	32.4	1.2	3.8	0.28
$P2 \times P4$	15	3.1	32.1	1.5	2.4	0.37
$P2 \times P5$	20	3.2	32.1	1.8	3.4	0.43
$P2 \times P6$	17	3.7	29.6	1.5	4.1	0.54
$P3 \times P4$	20	2.3	33.0	2.1	3.7	0.43
$P3 \times P5$	16	3.2	32.9	2.0	2.9	0.38
$P3 \times P6$	20	3.7	29.7	2.1	4.1	0.57
$P4 \times P5$	20	4.3	33.6	2.0	2.8	0.52
$P4 \times P6$	20	4.4	29.0	2.0	3.8	0.41
$P5 \times P6$	19	5.8	22.2	2.4	3.4	0.74
Minimum ^g		0.0	15.9	0.0	0.1	0.00
Maximum ^g		11.0	45.9	13.5	52.0	0.85
Mean		3.8	30.9	2.1	3.5	0.44
SE ^h		0.062	0.150	0.150	0.322	0.025
LSD _{0.05ⁱ}		0.127	0.304	0.303	0.650	0.050
Number of F_1 ^j		261	255	272	272	272

^a Number of genotypes evaluated per F_1 family; ^b Total carotenoid content ($\mu\text{g g}^{-1}$); ^c Dry matter content of roots (%); ^d Fresh root weight (kg plant^{-1}); ^e Fresh shoot weight (kg plant^{-1}); ^f Harvest index; ^g Values based on all F_1 genotypes evaluated; ^h Standard error; ⁱ Least significant difference at 5% confidence level; ^j Number of F_1 genotypes evaluated: reduction from the total population (272) indicates proportion of genotypes whose roots were not sufficient for measuring TCC and/or DMC.

DMC and TCC for all genotypes evaluated, with a negative correlation observed between the two traits.

Analysis of variance

Based on mean squares (MS), differences in performance of families were very significant ($P < 0.01$) for all the studied traits (Table 4). Mean squares of the crosses by environment interaction were significant for DMC, FRW and FSW, but such interaction was non-significant for TCC and HI. Meanwhile, GCA effects were highly significant ($P < 0.001$) for TCC, very significant ($P < 0.01$) for DMC, significant ($P < 0.05$) for HI, but non-significant for FRW and FSW. SCA effects were highly significant for FRW and FSW, very significant for DMC and HI and non-significant for TCC. The coefficient of variation (CV) associated with traits ranged from 7.5% for TCC to 21.2% for HI; these are within the acceptable range, and thus give confidence in the generated datasets. TCC had the highest value for Baker's ratio (0.89) followed by DMC (0.69), while FRW had the lowest value (0.36). A Baker's ratio above 0.5 implies that additive genetic effects are proportionately more important than dominance effects (Baker 1978). When total SS were partitioned into SS due to parents (GCA effects) and interaction

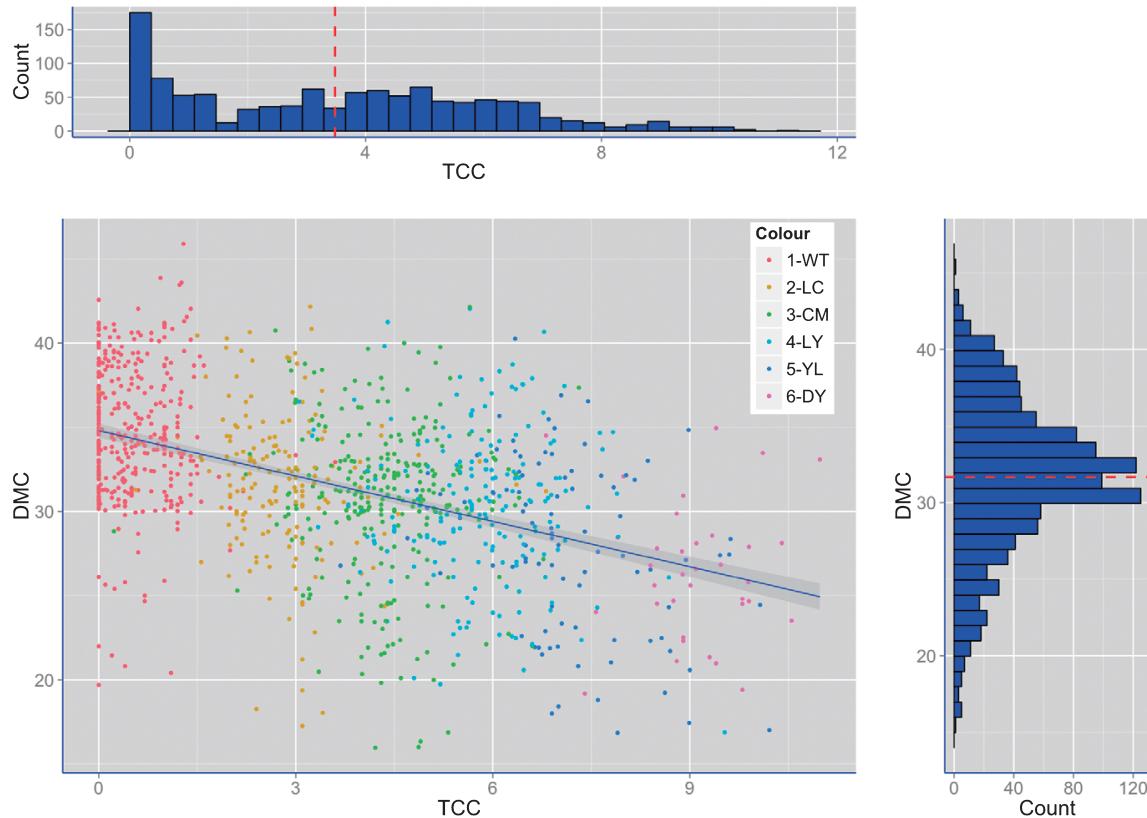


Fig. 1. Scatter plot and histograms of total carotenoids content (TCC) vs. dry matter content (DMC). Color represents pigmentation of the root parenchyma such that 1-WT = white; 2-LC = light cream; 3-CM = cream, 4-LY = light yellow; 5-YL = yellow; 6-DY = deep yellow; red lines on histograms indicate means.

Table 4. Mean squares of crosses and combining ability effects of five traits evaluated in 15 F₁ families and six parents at two locations

Source of variation	DF ^a	TCC ^b	DMC ^c	FRW ^d	FSW ^e	HI ^f
Environment (E)	1	0.03	9.31**	4.63***	31.29***	0.13**
Crosses (C)	14	4.23***	16.87**	1.75**	2.73**	0.07**
C × E	14	0.05	3.09***	0.26*	1.12*	0.01
GCA ^g	5	42.26***	16.66**	2.86	3.85	8.49*
SCA ^h	14	10.81	15.24**	10.30***	12.66***	16.21*
GCA × E	5	0.06	15.71***	0.73*	9.08**	0.05
SCA × E	14	1.01	6.47*	8.81*	15.95**	0.48*
% SS ⁱ due to GCA	71.31	64.34	21.29	12.84	51.27	
% SS due to SCA	24.48	31.28	56.84	48.11	28.51	
Residual	34	0.02	0.36	0.36	1.66	0.01
CV ^j (%)		7.50	16.31	11.21	19.42	21.20
Baker's ratio		0.89	0.69	0.37	0.38	0.56

^a Degrees of freedom; ^b Total carotenoid content ($\mu\text{g g}^{-1}$); ^c Dry matter content (%); ^d Fresh root weight (kg plant^{-1}); ^e Fresh shoot weight (kg plant^{-1}); ^f Harvest index; ^g General combining ability; ^h Specific combining ability; ⁱ Sum of squares; ^j Coefficient of variation. *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

between parents (SCA effects), the GCA effects accounted for more than 64% of the total variation expressed by the families for TCC and DMC, while FRW and FSW contributed less than 22% of variability in GCA effects. The SCA effects were important for FRW and FSW.

Table 5. GCA effects of cassava progenitors used in a 6×6 diallel analysis of five traits

Progenitor	TCC ^a	DMC ^b	FRW ^c	FSW ^d	HI ^e
NASE 3	-1.59***	1.75***	-0.11	0.38*	-0.12
CPCR24B-10	-0.38*	0.87**	-0.42**	-0.14*	-0.04
MH05-2870	-0.27	0.82**	-0.33*	0.63**	-0.05
MH05-0233	0.03	-0.17*	-0.06	-0.63**	0.03
CPCR15B-26	0.28*	0.44**	-0.03	-0.34**	0.02
MH02-073HS	1.93***	-3.72***	0.95**	0.12	0.16*
LSD _{0.05} ^f	0.253	0.607	0.605	1.300	0.020
SE ^g	0.041	0.097	0.097	0.208	0.014

^a Total carotenoid content ($\mu\text{g g}^{-1}$); ^b Dry matter content (%); ^c Fresh root weight (kg plant^{-1}); ^d Fresh shoot weight (kg plant^{-1}); ^e Harvest index; ^f Least significant difference at 5% confidence level; ^g Standard error. *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Combining ability and estimates of genetic parameters

Traits evaluated in this study were measured with preference for high scores (i.e. higher positive values for combining ability estimates were preferred for each of the evaluated traits). Genotype MH02-073HS, with the highest TCC, showed the highest GCA effect of 1.93 for the trait (Table 5). On the other hand NASE 3, a white-fleshed progenitor with negligible TCC, had significant negative GCA effect of -1.59 for TCC. Negative GCA's for TCC indicate unsuitability of specific progenitors as combiners when targeting

Table 6. SCA effects for a 6×6 diallel analysis of five traits evaluated at two locations in Uganda

Family	TCC ^a	DMC ^b	FRW ^c	FSW ^d	HI ^e
P1×P2	0.77	1.60*	-0.43	-0.49*	-0.04
P1×P3	-0.62	-1.17*	-0.87*	0.03	0.06
P1×P4	-0.98	-1.09	-0.23	0.12	-0.09
P1×P5	1.65*	1.22*	1.90**	0.16	-0.10
P1×P6	-0.82	-0.56	-0.37	0.17	0.16
P2×P3	-0.04	-0.43	-0.33	0.02	-0.25*
P2×P4	0.46	-0.04	0.52	0.06	-0.08
P2×P5	-0.18	-1.34*	0.83*	0.20	0.32*
P2×P6	-1.01*	0.21	-0.58	0.22	0.05
P3×P4	1.00*	0.54	0.71*	-0.04	0.12
P3×P5	-0.19	0.16	0.00	0.03	0.24*
P3×P6	-0.15	0.90	0.49	-0.04	-0.16
P4×P5	-1.87*	0.55	-2.09**	-0.08	-0.18
P4×P6	1.39*	0.04	1.09*	-0.05	0.22*
P5×P6	0.59	-0.59	-0.64	-0.31*	-0.28*
SE ^f	0.77	1.60*	-0.43	-0.49*	-0.04

^a Total carotenoid content ($\mu\text{g g}^{-1}$); ^b Dry matter content (%); ^c Fresh root weight (kg plant^{-1}); ^d Fresh shoot weight (kg plant^{-1}); ^e Harvest index; ^f Standard error. P1, P2, P3, P4, P5 and P6 are progenitors defined in **Table 2**. *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

high carotenoid content in the progeny. However, NASE 3 was the best general combiner for DMC with positive and significant GCA of 1.75, while progenitor MH02-073HS had negative and significant GCA of -3.72 for DMC. Only progenitor MH02-073HS had positive and significant GCA for FRW. Meanwhile, MH02-073HS was the best general combiner for HI, with a GCA effect of 0.16.

Three families (P1×P5, P3×P4 and P4×P6) showed positive significant SCA effects for TCC (**Table 6**). The other 12 families had either negative or non-significant positive SCA effects. Overall, there was high variation in SCA effects for DMC, with five families showing positive significant SCA effects. For this trait, family P1×P2 had the most positive and significant SCA effect of 1.60. For FRW, there were four F₁ families with positive significant SCA effects, with P1×P5 as the best specific combination. Meanwhile, none of the 15 F₁ families showed positive significant SCA effects for FSW. On the other hand, three families (P2×P5, P3×P5 and P4×P6) had positive and significant SCA effect for HI.

Phenotypic correlation among traits

The most positive and highly significant phenotypic correlation was between root flesh color (RFC) and TCC, with a coefficient of 0.94 (**Table 7**). This relationship indicated that higher intensities of root pigmentation reflected higher levels of TCC in roots. There was negative and significant correlation ($r = -0.44$) between the RFC and DMC. Similarly, a negative correlation was noted between TCC and DMC ($r = -0.45$). These relationships indicate that cassava roots with higher levels of TCC have low DMC. HI correlated positively and significantly with FRW but had significantly negative correlation with FSW, which is expected, given

Table 7. Phenotypic (lower diagonal) and genetic (upper diagonal) correlation coefficients for six traits in 6×6 diallel families evaluated at two locations in Uganda

Trait	RFC ^a	FRW ^b	DMC ^c	FSW ^d	TCC ^e	HI ^f
RFC		0.04	-0.62**	0.31	0.87***	0.02
FRW	0.14		-0.23	-0.03	0.83**	0.81**
DMC	-0.44**	0.07		0.08	-0.82**	-0.08
FSW	-0.02	0.07	-0.01		-0.05	-0.13
TCC	0.94***	0.132	-0.45**	-0.02		0.86**
HI	0.09	0.57**	0.09	-0.47**	0.08	

^a Root flesh color; ^b Fresh root weight; ^c Dry matter content; ^d Fresh shoot weight; ^e Total carotenoid content; ^f Harvest index. *, ** and *** significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

that HI is derived from the two traits. The most positive and highly significant genetic correlation was between RFC and TCC ($r = 0.87$). DMC had significant negative genetic correlation with TCC ($r = -0.82$). These genetic correlations (RFC with TCC and DMC with TCC) were consistent with patterns of phenotypic correlations between the same trait pairs.

Discussion

TCC in the F₁ progeny evaluated for this study varied from 0.0–11.0 $\mu\text{g g}^{-1}$, with a mean of 3.8 $\mu\text{g g}^{-1}$. Values for TCC in the progenitors varied from 0.2–10.4 $\mu\text{g g}^{-1}$. The observed TCC mean for the F₁ progeny was comparable to 3.6 $\mu\text{g g}^{-1}$ and 5.0 $\mu\text{g g}^{-1}$ reported, respectively, by Maroya *et al.* (2012) and Ssemakula and Dixon (2007) for breeding populations evaluated at IITA, but quite lower than the mean (14.7 $\mu\text{g g}^{-1}$) reported for populations at CIAT (Ceballos *et al.* 2013). The high level of TCC in cassava breeding populations at CIAT is a result of 10-year cyclic selection process imposed to primarily advance carotenoid-rich clones, targeted to attain levels above 15 $\mu\text{g g}^{-1}$. The population evaluated in the current study arose from a single round of recombination and fell short of this nutrient level targeted by HarvestPlus program (Pfeiffer and McClafferty 2007). It is worth noting that this study was strategically designed to generate pVAC clones expressing both CMD and CBSD resistance by including the white-fleshed NASE 3 as a progenitor. Nonetheless, the wide level of segregation for TCC observed in the current breeding population could provide a basis for implementing a recurrent selection scheme for developing cassava varieties with increased provitamin A carotenoid content in future. The rapid cycling scheme used by CIAT for increasing carotenoids content in the roots (Ceballos *et al.* 2013) could be adopted for this endeavour. With this scheme maximum carotenoids levels was increased from 10 to 26 $\mu\text{g g}^{-1}$ within a period of nine years.

DMC varied from 15.9–45.9%, with a mean of 30.9%. The mean DMC in the pVAC populations developed in this study is somewhat below the DMC levels (mean = 35%) of most improved white-fleshed varieties currently grown by farmers in Uganda (Kawuki *et al.* 2011). Importantly, some

of the carotenoids-rich genotypes evaluated here had higher DMC than that of varieties commonly grown by farmers, for example, 81 genotypes had DMC values $\geq 35\%$. These genotypes are of interest to breeding, as they qualify to be used as progenitors for the next round of recombination. High DMC in cassava roots is a trait highly preferred by farmers in SSA (Njukwe *et al.* 2013, Tumuhimbise *et al.* 2012). To enhance adoption of pVAC varieties in future, breeders will need to focus deliberate efforts on increasing DMC in the carotene-rich breeding populations. Fresh root yield is another critical trait that influences adoption of new varieties by farmers; however, in the current study, this trait was measured in form of FRW and HI. FRW potential was assessed on plant basis and it varied from 0 to 13.5 kg plant $^{-1}$. Genotypes with the ability to yield ≥ 3 kg plant $^{-1}$ would be preferred for cultivation, as current varieties popularly grown by farmers in Uganda have average yield of 25 t ha $^{-1}$ (2.5 kg plant $^{-1}$) (Kawuki *et al.* 2011). However, such genotypes identified from the current study will require further verification using larger plot sizes.

Two phenotypic correlations in this study are of special importance for developing pVAC cassava varieties. Firstly, the strong positive correlation between root flesh color and TCC is a good incentive for screening large early-generation breeding populations by visual assessment. At such stage of breeding, genotypes with higher intensity of root pigmentation can be selected for advancement, which saves time and the high costs associated with quantification of carotenoids. Iglesias *et al.* (1997) and Chávez *et al.* (2000) have previously demonstrated the effectiveness of using root color to select for high carotenoid content in cassava, especially if increasing carotenoids content is the sole breeding objective. However, Sánchez *et al.* (2014) suggest that the intensity of pigmentation, though useful for identifying high pVAC clones, may result in lower DMC. Perhaps a simplistic explanation is that high DMC has lots of white starch which tends to dilute the intensity of pigmentation. Thus, it would be important to make a mild initial selection based on color intensity and then a stronger one based on quantified carotenoids levels.

Continuous selection for both DMC and carotenoid content in Latin America has been underway much longer than in Africa and weak initial negative correlations could have been broken during the several cycles of recombination. For example, Ceballos *et al.* (2013) reported an interesting result of increased pVAC levels in breeding populations upon which several cycles recurrent selection were imposed for high levels of carotenoids content, yet DMC increased along with this selection strategy. In that study, the authors suggested that continuous recombination in a recurrent selection scheme could generate clones that combine high levels of both DMC and TCC, with values of up to 37% and 25 µg g $^{-1}$, respectively. Compared to other crops, Ortiz *et al.* (2014) reported a strong negative correlation between carotene content and fruit DMC in butternut squash (*Cucurbita moschata* D.), which is similar to reports by Vimala *et al.*

(2011) for studies on sweetpotato (*Ipomea batatas* L.). As further studies are undertaken, more useful genetic information relevant for cassava breeding will be generated and hopefully, this discrepancy clarified. For now, it is important to continue to pursue this approach of combining both traits.

Interpretation of the genetic nature of traits evaluated in this study was based on the mean squares for GCA and SCA effects. The proportion of SS for crosses explained by GCA components gives an estimate of the relative importance of additive effects in expression of traits (Falconer and Mackay 1996). GCA accounted for a significantly larger SS than SCA for TCC, DMC and HI, explaining more than 64% of the total variation for DMC and TCC. These results suggest that additive gene effects are more important in controlling accumulation of TCC and DMC in cassava. Similarly, 51.3% of the variation in HI was attributed to GCA. The relative importance of additive genetic factors for TCC, DMC and HI was reflected by the higher Bakers's ratios for these traits. Baker's ratio above 0.5, as was the case with these three traits, means these traits are under the control of additive genetic factors. The implication of these findings for cassava breeding is that a recurrent mass selection method can be an efficient breeding method for improving TCC and DMC, as it would enhance the exploitation of additive genetic effects for the traits (Ceballos *et al.* 2013).

FRW had non-significant GCA effects, suggesting that this trait is largely under control of non-additive genetic factors. This deduction is consistent with previous reports by Calle *et al.* (2005), Zacarias and Labuschagne (2010) and Kulembeka *et al.* (2012). In this case, breeders targeting the increase of fresh root yield in cassava would consider crossing progenitor combinations with superior SCA for FRW, a strategy that would increase the chances of selecting high-yielding clones from the segregating progeny (Chipeta *et al.* 2013). However, the relatively high importance of GCA effects for HI suggests a possibility of increasing FRW by crossing progenitors with high HI. Although Ojulong *et al.* (2010) demonstrated the effectiveness of using progenitors with high HI for improving FRW in cassava, the practice does not necessarily result in gains for root yield. In reality, it is common to find a genotype with low plant vigor (low foliage weight) showing a high HI when its actual root yield is very low (Hay 1995). Perhaps it would be a better practice to use HI as a complementary trait to FRW when selecting for high yield in cassava, which agrees well with suggestions by Hay (1995) that selections based on HI alone can be less effective.

Generally, progenitors with higher levels of TCC showed positive GCA, suggesting their contribution towards enhancing TCC in the progeny. Progenitor MH02-073HS had the highest positive and significant GCA effect for TCC, FRW and HI. Therefore, an appropriate breeding design would be to cross MH02-073HS to a genetic background of high DMC to increase chances of generating clones that accumulate favorable alleles for expressing higher levels of both traits (Ceballos *et al.* 2013). Similar relative importance

of GCA in inheritance of carotenoid content has been reported in *Cucumis sativus* L. (Navazio and Simon 2001) and *Zea mays* L. (Senete *et al.* 2011), suggesting the trait is controlled by genes that act additively. The highest negative GCA effects for TCC shown by NASE 3 is unsurprising, given it is a white-fleshed genotype with negligible levels of TCC. Conversely, the same genotype had the highest positive GCA effect for DMC, which also correlates well with its high level of DMC. NASE 3, also referred to as TMS 30572, is an IITA bred variety that is characterized with high levels of DMC (mean of >35%). This clone was officially released in Uganda in the 1990s and has remained popular among farmers, largely because of the high DMC and tolerance to both CMD and CBSD.

The non-significant SCA effects for TCC indicate that SCA is less important than GCA for inheritance of TCC in cassava, which is similar to deductions made by Senete *et al.* (2011) for inheritance of carotenoids in maize. Meanwhile, five F₁ families with positive and significant SCA effects for DMC could present possibility for generating clones with high DMC when such genotypic combinations are used for hybridization. Overall, SCA effects were more important than GCA effects for inheritance of fresh root yields, which agrees with previous reports by Kulembeka *et al.* (2012) and Tumuhimbise *et al.* (2014).

In summary, this study reports on a pioneer effort to breed cassava for high pVAC content, targeting to benefit the entire eastern Africa. Both breeding products in the form of genotypes and genetic information have been generated, all of which are useful for follow-up studies and future breeding programs. The generated datasets suggested that GCA effects are more important than SCA effects in the genetic control of carotenoid content in cassava, indicating that additive genetic effects largely control inheritance of carotenoids in cassava. The additive nature of carotenoids content provides scope for its improvement under the recurrent selection scheme. The negative correlation between root carotenoid content and DMC is important situation to deal with as a matter of priority. In particular, future breeding efforts that can uncover the genetic basis of the negative correlation between DMC and TCC are warranted. A breakthrough in this case could offer more realistic prospects for developing pVAC varieties acceptable to farmers. Such a breakthrough can be attained by at least two different approaches (that can hopefully be pursued together): 1) introducing germplasm from Latin America for combining high TCC and DMC and 2) strengthening the selection of segregating progenies based on the quantification of carotenoids as opposed to visual pigment selection.

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