Preliminary study of inheritance of the carotenoids content in roots from cassava (*Manihot esculenta* Crantz) segregating populations

Estudio preliminar de herencia del contenido de carotenoides en raíces de poblaciones segregantes de yuca (*Manihot esculenta* Crantz)

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Abstract

Vitamin A deficiency (VAD) is a major problem with huge public health implications. One strategy to overcome this problem is the development of varieties with increased levels of pro-vitamin A carotenoids. Cassava is a relevant crop in many regions of the world where VAD is prevalent. Significant progress has already been achieved increasing the content of total carotenoids (CTC) in cassava roots. However, little is known on the inheritance of this trait in cassava. In this study the segregations for CTC in several full-sib and self-pollinated (S_1) families were analyzed. Parent-offspring regression was used to estimate heritability, which was found to be high (>0.60). The analyses of segregations did not allow for the identification of simple Mendelian patterns that could explain the variation in CTC in every family analyzed. However, enough evidence has been generated for a hypothesis that few (2-3) major genes control most of the variation in CTC but their action is modified by few minor genes. Mounting evidence was also found that at least one single dominant gene may inhibit carotenoids accumulation in the roots.

Key words: Carotene, cassava roots, genetic variability, inheritance.

Resumen

La deficiencia de vitamina A es un problema de enormes consecuencias en la salud pública de muchos países en desarrollo. El perfeccionamiento y difusión de variedades con altos contenidos de carotenoides provitamina A es una estrategia para resolver este problema. La yuca es un cultivo relevante en muchas regiones del mundo cuyos habitantes padecen deficiencia crónica y generalizada de vitamina A. A pesar de que se ha logrado incrementar significativamente el contenido total de carotenoides (CTC) en raíces de yuca mediante el mejoramiento genético, es poco lo que se conoce sobre la herencia de CTC. En este estudio se analizaron numerosas familias de hermanos completos y S_1 (resultantes de autofecundaciones). La heredabilidad, estimada por regresión padre-progenies, resultó ser alta (> 0.60) y el análisis de las segregaciones no permitió definir patrones de segregación mendeliana simple que explicaran la variación en todas las familias. Sin embargo, hay suficiente evidencia para plantear la hipótesis de que la herencia es relativamente simple y depende de dos o tres genes mayores, cuya expresión es afectada por unos pocos genes modificadores menores. Se propone que al menos uno de los genes mayores inhibiría la acumulación de carotenoides.

Palabras clave: Caroteno, herencia, raíces de yuca, variabilidad genética.

Introduction

Cassava (*Manihot esculenta* Crantz) is a native crop from tropical America which consumption has been extended to Africa and Asia. This crop is characterized for its contrasting uses from food security till being a competitive and trustable source for starch, animal food and ethanol industries.

Deficiency of vitamin A (DVA) constitutes one of the main nutritional limitations in several human populations in developing countries (West, 2003). DVA is a serious public health problem in 37 countries and affects a considerable percentage of the population in regions where cassava is grown (WHO, 1995). An strategy to solve this problem is to improve the nutrient quality of crops using genetic breeding to increase the levels of the most important nutritional factors, by means of selection and recombination (Underwood, 2000, Chávez *et al.*, 2005; Morillo-C. *et al.*, 2011). Among the numerous carotenoids, β-carotene is the one with the highest vitamin activity and the most prevalent in roots of yellow cassava (Ceballos *et al.*, 2012a). Developing cassava varieties with higher content of total carotenoids (CTC) is not only offering benefits for human health but also for animal feed (Posada *et al.*, 2006).

Heritance studies of CTC in cassava roots are scarce and refer more to variation in root color than to nutrient quality (Hershey and Ocampo, 1989; Iglesias *et al.*, 1997). The objective of this study, based on a doctoral thesis (Morillo-Coronado, 2009), was to develop CTC segregant populations, determine variability in term of content of these pigments in those populations and thus, generate information to understand better the heritability of this character.

Materials and methods

Crosses to produce botanical seeds, field evaluations and carotenoids quantifications were performed at the International Center for Tropical Agriculture (CIAT), Palmira, Colombia.

Plant material

In the work to increase cassava CTC it was generated a high number of full-sib families (FHC). For this, a visual inspection of roots produced by each genotype was done for selection of those with an intense pigmentation to quantify CTC. However, occasionally all the FHC individuals were analyzed allowing genetic studies. In this study are presented the results of three FHC groups obtained through the years and identified as **first**, **second** and **third generation** of FHC. Among the families in the first generation three were selected which offered a broad variation range for CTC. For each of these families four individuals were selected with very low, intermediate and very high carotenoids levels. These individuals were cloned and growth on crossing nurseries to produce self-pollinating seeds (S_1). As result, 12 S_1 families were generated.

Two elite clones adapted to the Colombian Caribbean region (MTAI8 and CM4919-1) were self-pollinated as well, these are not related to the previously described materials. These two S_1 families were generated to perform different genetic studies, including analysis of CTC.

Plant material growth in the field

Obtained seeds (both from FHC and self-pollinating) were germinated and seedlings were transferred to the field following the normal protocols used by the cassava genetic breeding program of CIAT (Ceballos *et al.*, 2012b, 2012c). The management of the materials in the field included established practices of irrigation, fertilization and weed control. Plants were harvested between 10 and 11 months after field transfer. Between two and five roots of each genotype were randomly selected with the only criterion of being free of mechanical or insect damages and without apparent rotting. From each one of them the proximal and distal parts were discarded, the remaining fractions were cut in longitudinal quarters. From each root two opposite quarters were selected, chopped and mixed to extract three samples: one of 5 g for carotenoids analysis, other of 50 g to calculate dry matter percentage and the last one of 30 g as backup copy during the analyses (Ortiz *et al.*, 2011). This last sample was stored in conic bottom tubes of 50 ml on a freezer (-80°C).

Carotenoids extraction

The carotenoid extraction suggested by Safo-Katanga *et al.* (1984) was modified using petroleum ether (Chávez *et al.*, 2005; Ortiz *et al.*, 2011, Ceballos *et al.*, 2012a). Quantification was done by visible spectrophotometry using a Shimadzu UV-VIS 160[a] spectrophotometer. Detection was done at 450 nm (Rodríguez-Amaya 2001; Rodríguez-Amaya y Kimura 2004). CTC was estimated using the following formula:

$$\mu g/g = \frac{(A \; x \; final \; volume \; x \; 10^4)}{(A \; 1 \; cm - 1\%) \; x \; sample \; weight}$$

where:
A = Absorbance at 450 nm.
Final volume = Volume in milliliters of the solution before the reading.
A 1cm-1% = Coefficient of absorption of β–carotene in petroleum ether = 2592.
Sample weight = in grams.

All the values presented in this study are related to carotenoids concentration based on fresh weight.

Dry matter content

To estimate dry matter content, 50 g of finely chopped fresh roots were dry out on an oven at 60 °C for 24 h. dry matter was expressed as percentage of dry weight in relation to fresh weight.

Statistical analysis

For quantitative data (measures of central tendency, variation and deviation) descriptive statistics and frequency histograms were used for a better analysis of the information. Lineal regression analysis was used to estimate CTC heritability by means of the parent-offspring regression (Hallauer and Miranda, 1988; Lynch and Walsh, 1998). It has been demonstrated that the regression coefficient, when associating the phenotypical mean of the parents with the FHC progeny that they generate, is a suitable estimator of narrow-sense heritability (s^2_A/ s^2_F, where s^2_A is the additive variance and s^2_F is the phenotypic variance). In the case of self-pollinating families, there is only one parent and the regression is performed using the value of this sole parent. In this case, the meaning of the regression coefficient, in terms of quantitative genetics, is slightly different than in the case of FHC and results on a conservative estimation of heritability. However, if it is accepted that the allelic frequencies $p = q = 0.5$ or that there is no dominance, the meaning of the regression coefficient is similar in S_1 and FHC (Hallauer and Miranda, 1988).

Results

Several families from the first generation of FHC were evaluated in this study, but some of them had few individuals (less than eight), therefore they will not be presented in this article (Table 1). Several cassava materials flower late and produce few flowers, comprising one of the main constrains for genetic breeding and thus, production of segregant material is not ensured. In some cases, absence of flowering totally prevents crossings (Ceballos et al., 2012b; 2012c), problem that restrict the number of families with enough representative individuals.

Quantitative data showed a broad distribution of CTC in roots of the 21 FHC for the first generation (678 genotypes in total) listed in Table 1. In this group of FHC the range of variation for CTC was between 0.14 and 11.16 µg/g (fresh weight), with an average of 2.65 µg/g. In Table 1 are displayed in bold the three FHC (CM9816, GM 708 and GM 893) selected to generate S_1 families from CTC contrasting genotypes that will be described later.

In Table 2 are shown the results of the second and third generation of FHC. In the second generation are included nine families represented by 294 genotypes. The range of variation for CTC was between 0.21 and 13.92 µg/g with an average of 5.71 µg/g. In the third generation only four families represented by 292 genotypes were evaluated (Table 2). CTC values varied between 0.56 y 26.30 µg/g, with an average of 6.07µg/g.

The best average of families in the first generation of FHC was 4.83 µg/g and was significant, whereas in the second generation the average increased to 7.27 µg/g and in the third one was 9.92 µg/g. The increments in

Table 1. Descriptive statistics of the first generation of full-sib families (21 families) of cassava evaluated for carotenoids content (µg/g of fresh weight). Bold text identifies the three selected families from which S_1 families were obtained.

Family	n	Carotenoids (µg/g)			Stan. Dev.	Asymmetry
		Minimum	Maximum	Mean		
CM9312	28	2.05	5.67	3.60	0.95	0.305
CM9319	18	0.44	7.61	3.22	1.70	0.523
CM9345	18	0.22	8.38	3.00	1.93	1.284
CM9816	**8**	**1.46**	**9.99**	**4.83**	**2.96**	**0.466**
GM708	**66**	**0.16**	**11.16**	**3.14**	**2.65**	**1.117**
GM716	67	0.30	4.78	1.73	1.13	0.382
GM725	34	0.44	5.92	1.81	1.32	1.214
GM734	66	0.44	8.45	3.62	1.74	-0.007
GM738	34	0.59	7.00	3.69	1.85	-0.029
GM739	82	0.36	6.23	2.35	1.15	0.198
GM740	41	0.44	7.98	3.10	1.97	0.762
GM742	29	0.73	6.90	2.82	1.68	0.648
GM809	39	0.17	2.98	1.42	0.93	0.098
GM811	13	0.44	3.54	1.69	1.12	0.192
GM820	12	0.45	3.44	1.52	1.23	0.780
GM833	10	0.49	5.48	1.86	1.57	1.603
GM837	23	0.14	4.20	1.67	1.32	0.268
GM849	38	0.21	8.45	2.16	2.18	1.297
GM890	20	0.19	6.78	2.46	2.08	0.738
GM893	**20**	**0.76**	**8.77**	**4.46**	**2.44**	**0.443**
GM894	12	0.17	3.16	1.56	1.06	-0.144

n = family size (number of individuals).

Table 2. Descriptive statistics for some root characteristics in full-sib families (second and third generations) of cassava evaluated for the total content of carotenoids (µg/g of fresh weight).

Family	n	Carotenoids (µg/g)			Stan. Dev.	Asymmet
		Minimum	Maximum	Mean		
			Second generation			
GM1546	10	2.61	11.06	7.27	2.81	-0.033
GM1547	49	2.12	12.44	6.47	2.27	0.828
GM1548	56	2.36	13.71	6.04	2.32	1.109
GM1549	12	0.21	5.42	2.32	1.53	0.801
GM1550	47	0.73	11.96	5.60	2.37	0.497
GM1551	56	0.38	13.44	5.16	2.58	0.780
GM1552	29	0.49	7.93	5.12	1.74	-0.724
GM1560	21	3.64	13.92	6.91	2.35	1.867
GM1561	14	3.00	12.18	6.49	2.66	1.152
			Third generation			
GM3465	43	1.24	3.70	2.34	0.66	0.408
GM4655	140	0.56	7.19	2.97	0.78	0.226
GM3732	37	3.14	26.30	9.92	4.18	1.423
GM3736	72	1.26	21.00	9.04	4.84	0.252

n = family size (number of individuals).

the best averages reflect the maximum value observed in individual genotypes in the first, second and third generation of FHC (11.16, 13.92 and 26.30 µg/g, respectively) and are an evidence in the genetic progress that could be obtained to increase CTC in cassava roots.

In Table 1 are also included the coefficient of asymmetry for each family. The average through the 21 FHC was 0.578, indicating that the distributions tend to have higher picks of frequency in low CTC values, with tails extended to the right on the distribution histograms, it means, to the zones with higher CTC values. To some extend this makes sense since there is a physical limit at the left of the histograms and the roots cannot have negative values for CTC. However, there are six families with very low coefficients of asymmetry (between -0.200 and 0.200) suggesting a more symmetric distribution. Asymmetries in the second and third generation of FHC are included in Table 2. The average value for the 13 families described in this Table was 0.660 and only one family (GM 1546) presents a value close to cero.

Results of the S_1 families are included in Table 3. The problems to get enough botanical seed in cassava are aggravated when seeking for self-pollinations due to the typical protogyny of this crop (Ceballos and De la Cruz, 2002). It is important to notice that the family AM700 only produced two individuals that could be evaluated. CTC values of the parents of each self-pollinating family are also shown in Table 3. There is a clear relation between these values and averages of the families generated by these individuals, except by the family AM700 that presented a limited number of individuals.

The range of variation observed in the analysis of segregant materials in the S_1 families is of particular interest. If inheritance is due to one or two genes, as initially postulated, when auto-pollinating materials of high or low CTC variation should be fixed for that trait with a drastic reduction in the range of variation, which might have happened only in the family AM697 (originated from a genotype with low CTC levels) in which any of its 38 individuals showed CTC levels higher than 1.0 µg/g. However, the AM689 family, also originated from an individual with low CTC levels and from the same FHC, had a broad range of variation was noticeable low (1.10 µg/g). The coefficient of asymmetry in this family is high

Table 3. Descriptive statistics for CTC (µg/g of fresh weight) in roots of S_1 families of cassava coming from selected genotypes of first generation FHC families. For each FHC genotypes were selected with high (A), intermediate (I) and low (B) values of CTC.

S_1 Family	Carotenoids on the parent	n	TC (µg/g)			Stan. Dev.	Asymmetry
			Minimu	Maximum	Average		
S_1 families derived from the full-sib family CM 9816							
AM 690	9.99 (A)	90	2.89	13.10	6.51	1.82	-0.599
AM 689	6.67 (I)	71	0.46	11.37	4.73	2.69	-0.045
AM 692	5.04 (I)	48	4.85	8.95	6.38	0.77	0.026
AM 691	1.87 (B)	73	0.40	5.89	2.34	1.18	0.892
S_1 families derived from the full-sib family GM 708							
AM 702	11.16 (A)	29	0.63	19.1	6.46	5.92	0.734
AM 700	7.32 (I)	2	2.01	2.79	2.40	0.55	n.d.
AM 698	0.54 (B)	34	0.30	6.56	1.10	1.25	3.298
AM 697	0.27(B)	38	0.26	0.89	0.54	0.15	0.34
S_1 families derived from the full-sib family GM 893							
AM 710	8.77 (A)	29	4.85	8.95	6.38	0.77	-0.299
AM 718	8.14 (A)	38	3.19	9.24	5.90	1.28	-0.025
AM 712	6.06 (I)	57	0.44	8.41	3.31	2.06	0.478
AM 720	2.04 (B)	40	0.14	9.72	2.28	2.22	0.432
S_1 families derived from elite clones MTAI 8 and CM 4919-1							
AM 320	2.14 (B)	177	0.37	8.10	2.22	1.37	1.084
AM 324	3.83 (B)	11	0.75	8.58	3.83	2.35	0.313

n = family size (number of individuals.

(3.298) suggesting a large concentration of frequencies for low CTC values and a dim and long tail to the right of histogram. In fact, this family had 34 individuals and only four of them had CTC levels higher than 2.00 µg/g.

In any of the cases the self-pollinating results of high CTC level genotypes suggest that the variation around the high CTC levels could be fixed (Table 3). It is noticeable the range of variation observed in the family AM702 (0.63 - 19.1 µg/g). Both families (AM710 and AM718) derived from individuals with high CTC levels from the FHC GM893 seem to have fixed the capacity to accumulate, at least, a minimum CTC level over 3 µg/g; something similar happened with the AM692 family.

In general, observed segregations in the S_1 families showed three types of behavior (Figure 1). In some families there is a clear asymmetry enriched in frequencies to the left of the histograms, with a drastic reduction of genotypes with intermediate and high CTC levels. In other cases (Figure 1), it is observed a more symmetric distribution with higher frequencies towards the intermediate CTC values. There is a third type of graphs in which a trimodal distribution is implied. Self-pollinating families from parents with low CTC values tend to produce several genotypes with 5-6 times higher CTC values than their parents. When the CTC values for the parents was intermediate or high, only few genotypes exceeded the parents' levels and the vast majority showed lower values. This explains the coefficient of asymmetry trend to show negative values only for families with parents of intermediate or high CTC levels. In Figure 2 is shown the observed segregation in the S1 family AM320, derived from the elite clone MTAI 8. A special emphasis in his family is

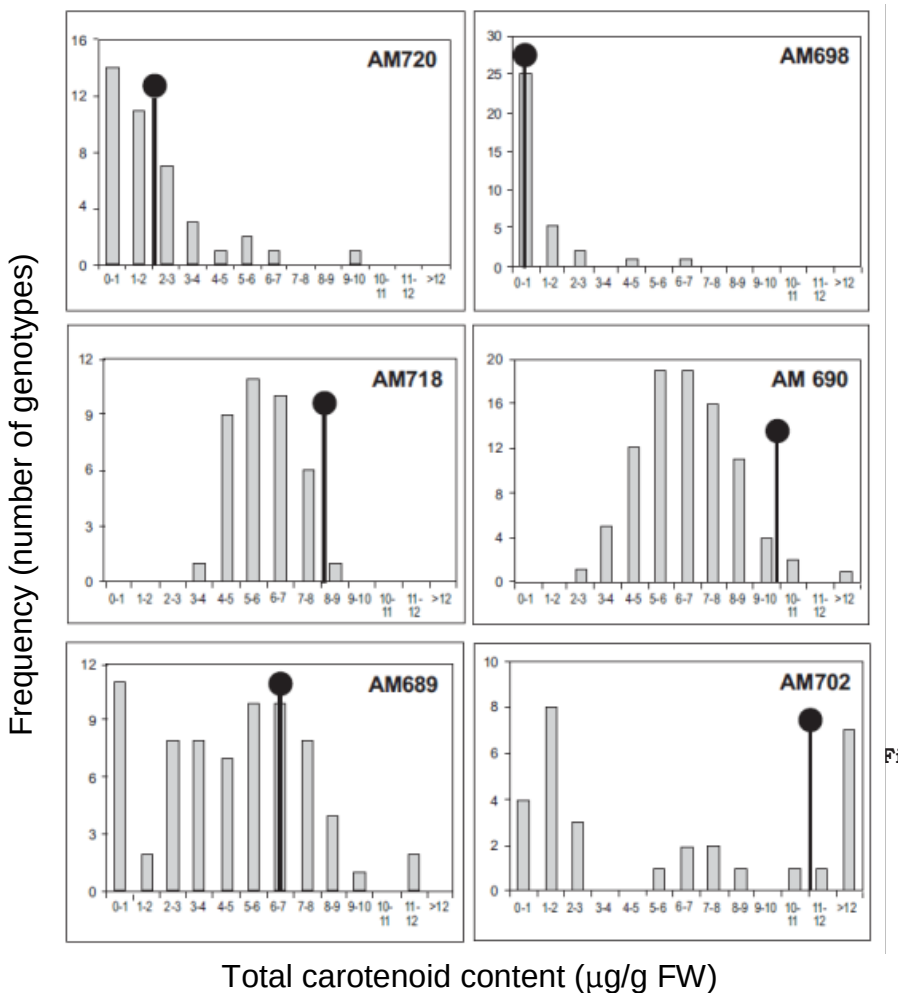

Figure 1. Histogram illustration of contrasting frequencies for total carotenoid content in cassava roots. This graphs were selected among the 12 self-pollinated families. Black circles indicate the CTC values of the parents of the respective families.

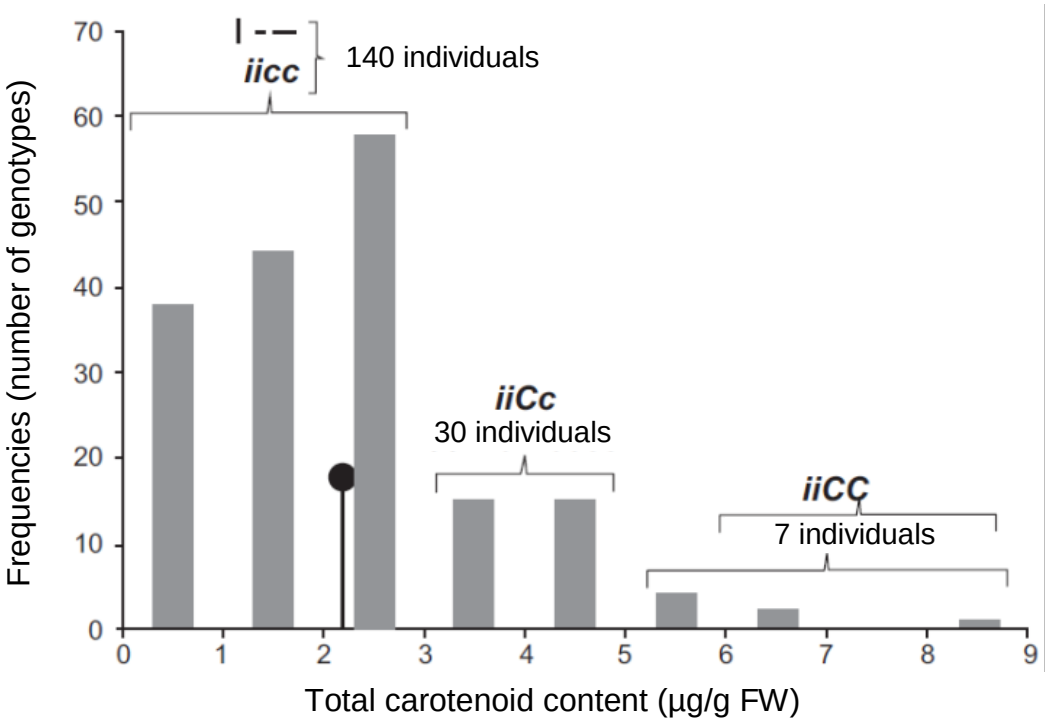

Figure 2. Histograms of frequency with the observed segregation in the S1 family of 177 individuals of cassava derived from the elite clone MTAI 8. Black circle denotes the parent CTC (2.14 µg/g fresh weight). The genotypes postulated for each class are also presented.

done because it was represented by 177 individuals, thus the results of this segregation are very solid.

Relation between parents' averages and their respective progenies, expressed by the lineal regression analysis, is a good estimator of heritance of characters. It has the advantage of taking into account the precision of performing phenotypic evaluations, the way the alleles are transmitted to the descendants, including potential linkage and, the genetic interactions (dominance and epistasis) in the final expression of characters. In this study, the parent progeny regression was made based on 36 observations (see Figure 3). These values are originated as follows: from the 21 FCH (first generation) in only 10 the CTC from their parents was known, thus, this generation contributes with 10 observations; all the second and third generation FHC families (nine and four families, respectively) were considered because their parents' CTC was known. From the 14 S_1 families listed in Table 3, AM700 was not considered, which has only two individuals, thus only 13 observations derived from this kind of family were considered.

Lineal regression coefficient (estimator of heritability) was 0.60 when values from HC and S_1 families were integrated. As the meaning of this coefficient in terms of quantitative genetics could be slightly different in the progenies of the HC or S1 families according to the importance of the no additive effects and the allelic frequencies, the heritability was estimated as well and the self-pollinating families were ignored, which made the heritability increased to 0.65. This support the concept that the estimation of heritability using the parent-progeny regression in families S_1 tends to be conservative (Hallaurer and Miranda, 1988). These values, relatively high, result being useful and explain how the recurrent selection of short cycles implemented at CIAT triplicate, on a ten years period, the maximum values of CTC in cassava roots found at the beginning of the project (Ceballos *et al.*, 2012c). All these observations are gradually converging to support the hypothesis that the genetic control for CTC in cassava

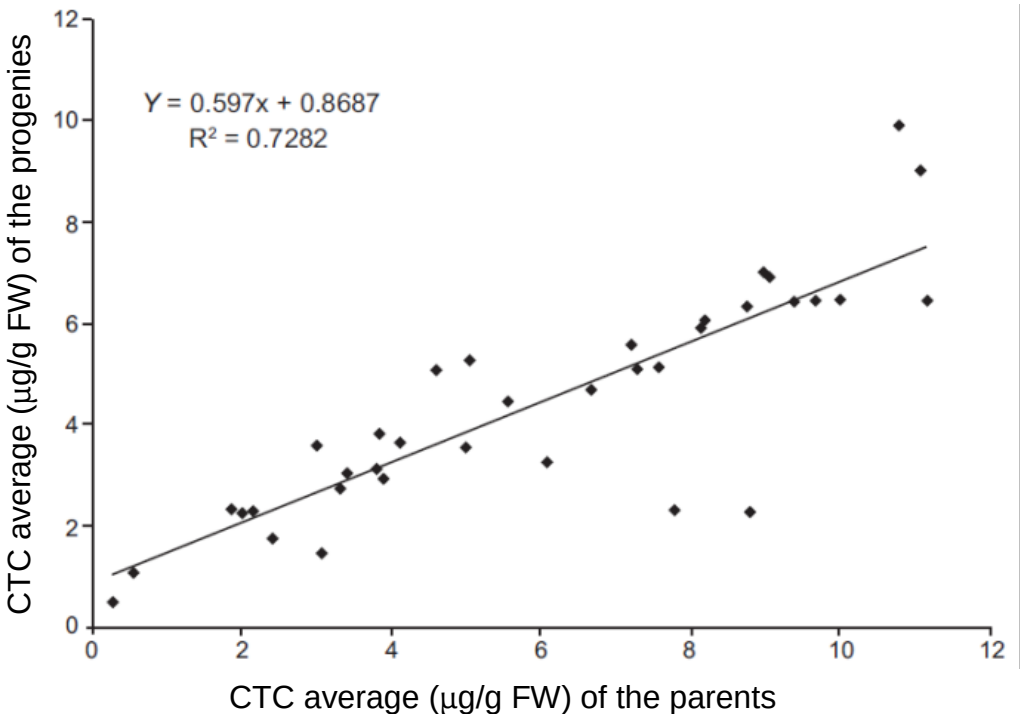

Figure 3. Lineal regression between CTC values or averages of the cassava parents and their respective progenies. The coefficient of regression is a good estimator of the character heritability.

roots is relatively simple and not much affected by the environment.

Even though the parent-progeny relationship shows a clear tendency, some points are interesting (Figure 3). In two cases the parents' averages varied between 8mg/g and 9 µg/g, while the respective progenies had averages of approximately 2 µg/g. for this cases some explanation can be proposed and the most obvious is a recessive behavior for CTC. This situation, however, is the opposite of what is suggested for the other analyzed materials. Although the caring measures were taken, other explanation is that problems with the pollinations originating these families happened; for example, the mistaken identification of one parent. It is also possible an overestimation in CTC quantification of the parents. One of the families is part of the second generation of full-sibs (GM 705- 5 x GM 708-63), while the other is part of the third generaion of full-sibs and is originated by the cross MBRA 496 x MBRA 1321.

It was not observed a relation between the four parents that originated these two families with abnormal behavior. The parents GM 705-5 and GM 708-63 were used in several ocasions to produce other FHC or self-pollinations. In the other cases this apparent recessive behavior is not observed.

Discussion

In initial basic genetic studies in cassava, there were identified genes for two characters: foliar lobe shape and root color. Graner (1942) and Jos and Hrishí (1976) demonstrated that segregation for wide or narrow leaf lobe is controlled by one gene, additionally affected by age and environment. Graner (1942) found that the dark color on the skin of the root is dominant over the light color. The reddish or greenish color of the leaf veins has also been used as genetic marker, but the number of genes controlling this character has not been studied (Kawano *et al.*, 1978). More recently, a normal Mendelian segregation was confirmed and a recovery in progenies of 25% with expression of the recessive character of starch without amylose from a 12,000 individuals population (Aiemnaka *et al.*, 2012).

Studies in different crops suggest that heritability for carotenoid content or parenchymal color in different roots and tubers is high (Jones 1977; Haynes *et al.*, 2011), depends on relatively few genes (two or three) (Fernandes Santos and Simon, 2006) and that carotenoid content tend to be dominant over absence of these pigments (Goldman and Breitbach, 1995). The relationship between yellow color and concentration of provitamin A compounds on cassava roots has been widely demonstrated (Hershey and Ocampo, 1989). To establish the hypothesis that a single gene controls the trait and that yellow is dominant over white, data from several crosses was used. Iglesias *et al.* (1977) suggested that carotenoids synthesis and accumulation in cassava roots could be regulated by two major genes. More recently, Akinwale *et al.* (2010) demonstrated that heritance for cassava root color could be controlled by two or more genes, without observing reciprocal effects. All the segregation studies mentioned are referred to the parenchymal color without studying carotene content.

Recently it was published the first study on molecular aspects of carotenoids synthesis and accumulation in cassava roots (Welsh *et al.*, 2010). It describes for the first time the PSY (phytoene synthase) regulation in cassava roots, which is dominant and affects carotene synthesis by different enzymatic activity efficiencies.

Results of the present study showed for the first time the estimation of heritability for CTC in cassava being relatively high (> 0.60), which is an important contribution to knowledge and explains the fast gain in increments of CTC in cassava roots obtained after short cycles recurrent selection in CIAT (Ceballos *et al.*, 2012c).

Results also suggested a relatively simple heritance but based on more than one genetic factor, which agrees with molecular studies based on mycrosatellites (Morillo *et al.*, 2011). Segregations observed in different families allow the postulation of a hypothesis on the genetic basis of CTC in cassava. This hypothesis is used to explain the segregation illustrated in Figure 2 and is based on the existence of two major genes (tentatively named ***I*** and ***C***). The ***I*** gen would be the dominant and inhibits carotenoids accumulation in cassava roots. From the metabolic point of view, it is not necessarily an inhibition that blocks carotenoids synthesis or accumulation. It is possible that the action mode of this gene is to divert the metabolic process to compounds different to carotenoids. Unpublished data (J. Arango Mejía, personal communication, 2012) suggest that in the case of carrot the overexpression of β-carotene hydroxylase (β-HYD) results on a reduction of the carotenoids content in this root, situation already demonstrated on wheat (Qin *et al.*, 2012). Other alternative could be degradation genes like CCD (carotenoid cleavage dioxygenase) that favor carotene metabolization to other compounds (xanthophylls). Future studies will confirm with more precision the nature of this recessive factor.

The second gene (***C***) would favor the carotenoids accumulation and might be related with *PSY*. It is postulated, therefore, that it has an additive mode of action. According to this hypothesis, MTAI 8 will be ***IiCc***. When an individual of this genotype is self-pollinated the progenies generated would segregate to produce the following phenotypic classes:

1. ***IICC***, ***IICc***, ***Iicc***, ***IiCC***, ***IiCc***, and ***Iicc***, that would tend to have a low carotenoids level as result of the dominant effect of the allele ***I***. accumulated frequencies of all these genotypes would be 12/16 (for the typical frequencies of a dihybrid Mendelian segregation).
2. ***iiCC***, having no inhibitory effect of *I* could accumulate carotenoids. Having the maximum number of alleles favoring carotenoids accumulation (***C***) it results in high CTC levels. The frequency of this genotype will be 1/16.
3. ***iiCc***, having no inhibitory effect of ***I*** it could accumulate carotenoids. Having only one allele *C* result in intermediate levels of CTC. The frequency of this genotype will be 2/16.
4. ***iicc***, while having no inhibitory effect of *I*, it will not accumulate carotenoids because there is no allele ***C***. The frequency of this genotype will be 1/16.

The expected frequency of individuals with low CTC (numerals 1 and 4 above) will

Table 4. Hypothetical explanation of the observed segregations in the S_1 families of cassava analyzed in this study. The second column shows the hypothetical genotype of the parent of each family and in parenthesis the CTC of that parent. In the following three columns are presented the frequencies for the three phenotypical classes (low, intermediate and high CTC levels), as well as the threshold between classes. In the last column is shown the respective chi-square test.

Family	Parent genotype	Observed frequencies (expected frequencies)			X^2 Test
		Low CTC	Intermediate CTC	High CTC	
	S_1 families derived from the full-sib family CM 9816				
AM 690	iiCc (10.0)	18 (<5.0)	54 (<8.0)	18 (>8.0)	3.6
AM 689	iiCc (6.7)	21 (<3.0)	35 (<7.0)	14(>7.0)	1.4
AM 692	iiCc (5.0)	13 (<5.0)	26 (<6.5)	9 (> 6.5)	1.2
AM 691	IiCC (1.9)	55 (<3.0)	18 (mayor 3)		0.01
	S_1 families derived from the full-sib family GM 708				
AM 702	iiCc (11.2)	12 (<2.0)	12 (<13.0)	6 (>13.0)	3.60
AM 698	IiCc (0.5)	30 (<2.0)	3 (<6.0)	1 (>6.0)	1.17
AM 697	II—(0.3)	38 (<2.0)			Perfecto
	S_1 families derived from the full-sib family GM 893				
AM 710	iiCC (8.8)	0 (<3.0)	1 (<5.0)	28 (>5.0)	n.d.
AM 718	iiCC (8.1)	0 (<3.0)	10 (<5.0)	28 (>5.0)	n.d.
AM 712	iiCc (6.1)	17 (<3.0)	27 (<6.0)	13 (>6.0)	0.72
AM 720	IiCc (2.0)	30 (<3.0)	6 (<6.0)	4 (>6.0)	1.29
	S_1 families derived from the elite clones MTAI 8 and CM 4919-1				
AM 320	IiCc (2.1)	140 (<3.0)	30 (<5.0)	7 (>5.0)	2.19
AM 324	iiCc (3.8)	3 (<3.0)	6 (<5.0)	2 (>5.0)	0.27

n.d.: Chi-square test cannot be performed because the expected value for the class is cero.

be13/16. The expected frequencies of individuals with intermediate CTC levels (numeral 3 above) and high (numeral 2 above) will be, respectively, 2/16 and 1/16. Chi-square test supports the basis of this hypothesis. In general, when this kind of work is performed it should be defined, maybe arbitrarily, the threshold to define low, intermediate and high CTC materials. However, the same thresholds have been applied to all the progenies derived from the same FHC (Table 4) with satisfactory results.

Carotenoids quantification is highly trustable and replicable. Nonetheless, it is possible to observe not expected variation in CTC in roots of the same plant (Ortiz *et al.*, 2011). This variation can be a problem in breeding programs because could lead to rejecting genotypes with high CTC or accept undesirable genotypes, but it is even more complicated when segregations are the subject of study because it generates results that are hard to explain.

Additionally to the two major genes proposed, there is, maybe, a third major gene and possibly an additional group of modifier genes that generate a quantitative variation towards the produced phenotypic classes by the major genes. It cannot be specified the number of minor genes but there is enough evidence to justify that they are few (possibly less than five). These hypotheses are based on the information presented in Table 4. The S_1 families derived from genotypes with the highest CTC (AM690 and AM702) required higher thresholds between the different phenotypic classes than in other related families.

Conclusions

- Narrow-sense heritability for CTC in cassava roots in high (> 0.60).
- By integrating the information generated in this study with observations and results

in the selection process for high CTC, is consolidated the idea that CTC depends on few major genes (two or three) and there is a group of modifier genes that alter the scales in which the major genes act. It is to highlight that the hypothetical model proposed is only a point to start and is mainly postulated to motivate future studies in order to validate and improve it.

- There is evidence that at least one of the major genes could have a recessive action possibly by processing carotenoids to other metabolic faiths, like the xanthophylls. Something similar could happen in the segregation of the MTA18 self-pollination.

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