

OUTPUT 1
Genetic base of cassava and related *Manihot* species evaluated and available for cassava improvement: higher nutritional quality.

The overall objective of this output is to generate genetic stocks and knowledge about genetic variability for nutritional quality traits in cassava. The main activities focus in developing and identifying cassava germplasm whose roots have higher carotene contents. Protein, Zn and Fe contents are also considered. The scope of research does focus on nutrients concentrations, related agronomic characteristics and the effect of processing. Related issues are the need for a better understanding of the biochemical and genetic basis of these high nutritional quality traits.

Because of the nature of the research described in this output, it is one of the many collaborative activities between projects **SB2** and **IP3**, as well as the **HarvestPlus** Challenge Program. To maintain some coherence through this report some of the activities reported herein may also be reported by **SB2** and/or **HarvestPlus**. Several scientific articles are currently under revision or have been accepted for publication in peer-reviewed international journals. Many of the activities and results related to Output 1, therefore, are going to be presented through the Materials and Method, Results and Discussion sections of the respective manuscripts. Since some of these manuscripts use data generated after many years of research, some results may involve data reported earlier. Since there are many references shared by the different manuscripts all references are pooled together at the end of Output 1.

Activity 1.1. Variation of quality traits in cassava roots evaluated in landraces and improved clones (Euphytica).

1.1.a Materials and methods

A total of 2457 cassava clones have been evaluated and a description of the origins of this germplasm is provided in Table 1.1. There were two types of clones, those produced from breeding projects at International Center for Tropical Agriculture (CIAT, Colombia), International Institute of Tropical Agriculture (IITA, Nigeria) or Rayong Experimental Station in Thailand, and clones from landraces from the germplasm collection held at CIAT.

Table 1.1. Summary of the origin of the cassava clones evaluated in one or more of the different analyses described in this article.

Origin	No.	Origin	No.	Origin	No.
CIAT's clones	337	Dominican Rep.	8	Paraguay	77
IITA's clones	4	Ecuador	77	Peru	217
Argentina	13	Fiji Islands	2	Philippines	7
Bolivia	3	Guatemala	31	Puerto Rico	10
Brazil	585	Indonesia	19	Thailand	9
China	4	Malaysia	31	USA	7
Colombia	720	Mexico	49	Venezuela	122
Costa Rica	32	Nigeria	4	Other	21
Cuba	45	Panama	23	TOTAL	2457

Because of limitation in the number of samples that can be analyzed at any given time and the impossibility of storing the roots, the evaluations were carried out through a period of four years since 1998 through 2001. Plants maintained at the *in vitro* germplasm collection were hardened in greenhouse conditions and, after two months, transplanted to the field. Evaluations were unreplicated, because of the lack of planting material and the time required to multiply it. Tissue samples from no less than three roots per accession were taken 10 to 11 months after transplanting. All plants evaluated were grown at CIAT station in Palmira (Valle del Cauca Department, Colombia).

Carotene concentration.

The extraction procedure outlined by Safo-Katanga et al. (1984) was modified by extracting root parenchyma with petroleum ether, as described and utilized by Iglesias et al., 1997. The modified protocol included several extractions with petroleum ether (35-65 °C). Approximately 5 g of tissue was obtained from representative and randomly selected roots from plants of each clone. The use of alternative solvents has been suggested more recently (Rodriguez-Amaya, 2001) and incorporated in more recent quantifications, which are not reported in this article. The quantification was done by visible spectrophotometry using a Shimadzu UV-VIS 160A recording spectrophotometer. Detection was done at $\lambda = 455\text{nm}$ (Rodriguez Amaya 1989; 1990; Scott & Hart, 1993).

Post-Harvest Physiological Deterioration (PPD).

Five commercially sized roots (minimum length 18 cm) were randomly chosen. Roots were analyzed using the method of Wheatley et.al. (1985), with one modification: prepared roots were stored under ambient conditions for 7 days instead of 3 days. The proximal and distal root ends were cut off and the distal end was covered with clingfilm. After one week, seven transversal slices, 2 cm thick were cut along the root, starting from the proximal end. A score of 1-10 was assigned to each slice, corresponding to the percentage of the cut surface showing discoloration (1=10%, 2=20%, etc). The mean score of PPD for each root was calculated by averaging the score across the seven slices.

Minerals concentrations.

The sampling procedure was the same as for the evaluation of carotene content. Roots were dried, ground to powder and sent to the Analytical Laboratory of University of Adelaide where the samples were analyzed by inductively coupled plasma atomic emission spectrometry. All sample processing was carried out to avoid as much as possible contamination from soil, which has mineral concentrations higher than that of vegetal tissues. Protein content was estimated by multiplying N concentrations by a constant of 6.25, although Hock-Hin & Van-Den reported in 1996 that in the case of cassava this figure is probably ranging from 4.75 to 5.87. The original conversion factor has been maintained to facilitate the comparisons with previous reports. N quantification was based on dried root flour. Therefore, HCN had already been released before the quantification and no nitrogen from cyanogenic compounds should have remained.

Dry matter content.

Dry matter content was estimated using the well-known specific gravity methodology (Kawano et al., 1987). Approximately five kilograms of roots were weighted in a hanging scale (WA). The same sample was weighted with the roots submerged in water (WW). Dry matter content was estimated with the following formula:

$$\text{Dry matter content (\%)} = \left\{ \frac{\text{WA}}{\text{WA}-\text{WW}} \right\} * 158.3 - 142$$

where WA= weight in the air and WW= weight in water.

Root coloration and other measurements.

A 1 to 9 scale for the visual estimation of root coloration was developed and printed for a uniform estimation of color intensity. The color of root parenchyma can vary from white, cream, yellow, and orange. Pinkish roots (score 9) have also been observed in cassava. Total and reducing sugars were estimated following the procedure outlined by Cronim & Smith in 1979. Cyanide potential (HCN) was quantified following the colorimetric procedure suggested by Essers et al. (1994).

1.1.b Results

Table 1.2 presents a summary of measurements for dry matter, HCN, total carotene for roots and leaves, as well as color, PPD and sugars in the roots. Descriptive statistics make use of all the data available for each variable. However, for the association between two traits, only data taken on the same roots for the traits whose association is analyzed were used.

The observed values for dry matter content and HCN in roots agree with those reported in the literature (Buitrago, 1990). The average for PPD was 24.47%, with individual values ranging from 0 to 100%. Distribution of PPD was asymmetrical with a longer tail to the right, and concentration of frequencies around the low-PPD values.

Table 1.2. Descriptive parameters for root traits of industrial relevance in accessions from the Cassava Germplasm Bank and the Breeding Project at CIAT.

Variable	Sample size (No.)	Minimum	Maximum	Average	Standard Deviation
Root dry matter (%)	2022	10.72	57.23	34.27	6.95
HCN (ppm)	2022	13.9	2561.7	263.7	324.2
Carotene (µg / g FT)	1789	1.02	10.40	2.457	1.351
Root color (1 to 9)	788	1	8	2.26	1.46
PPD (%)	1374	0	100	24.47	19.63
Total sugars (%)	1755	0.2	15	2.876	2.028
Reducing sugars (%)	1755	0.0	12.9	0.753	0.957

Carotenes in the roots and related traits.

Carotene content in the roots ranged from 1.02 to 10.40 µg / g fresh tissue (FT), demonstrating the potential of cassava clones with yellow roots to contribute overcoming VAD in regions of the world where this malady is a chronic problem. There was a clear asymmetrical distribution for carotene in the roots, which concentrated frequencies in the lower values to the left of the plot (Figure 1.1), and a long tale to the right (skewness value =

2.64). The visual scoring of root color, based on a sample of 788 clones also had a higher frequency of roots with light or white coloration, with fewer cases of roots with intense coloration (skewness = 1.73).

Table 1.3 presents the correlation coefficients among different root traits. There was a clear and positive association between carotene and HCN in the roots. This association, as suggested by Graham et al. (1999), is probably due to the fact that clones with yellow roots are commonly found in the Amazon basin, where highly cyanogenic (bitter) lines are also preferred. It is possible, however, to obtain clones with intense yellow coloration in the roots yet low levels of HCN. For instance, the elite clone CM 2772-3 developed for the Putumayo Department in Colombia's Amazon Basin, has yellow roots with a relatively high concentration of carotene (the average from two evaluations was 0.51 $\mu\text{g} / \text{g FT}$) and low level of HCN (93.5 ppm from the same samples where carotene were measured).

Table 1.3. Simple phenotypic correlation among relevant root traits in cassava. Between parenthesis the size of the sample utilized to measure the correlations.

	HCN (ppm)	Carotene ($\mu\text{g} / \text{g FT}$)	PPD (%)	Total Sugars (%)	Red. Sugars (%)
Dry matter content (%)	-0.222** (1374)	-0.078** (1315)	0.348** (1374)	-0.364** (1374)	-0.349** (1374)
HCN (ppm)	1.0	0.305** (1315)	-0.080** (1374)	0.167** (1374)	0.141** (1374)
Carotene ($\mu\text{g} / \text{g FT}$)		1.0	-0.123** (1315)	0.082** (1315)	0.052 ^{NS} (1315)
PPD (%)			1.0	-0.114** (1374)	-0.120** (1374)
Total Sugars (%)				1.0	0.595** (1374)
Red. Sugars (%)					1.0

** Significant

Phenotypic correlation between total carotene content in the roots and root color score (n=788) based on the visual scale was very high and positive ($\rho = 0.860$). This value demonstrates that the identification of cassava clones with high carotene density in the roots can be easily and effectively done through a visual evaluation of their parenchyma color. The higher the color intensity, the higher the amount of carotene present, supporting the reports by Iglesias et al. (1997) and Graham et al. (1999) but based on a considerably larger sample.

PPD and related traits

Because of the economic importance, the association between PPD and other traits was also analyzed. The correlation coefficient ($\rho = 0.348$) indicates that there is a positive association between dry matter content and PPD, further supporting previous reports (Jennings & Hershey, 1985; van Oirschot et al., 2000). This is an unfortunate situation because, in general, breeding projects look for higher dry matter content, which leads to a faster or more serious PPD. HCN does not seem to have a strong effect on PPD, whereas carotene seemed to reduce and/or delay the onset of PPD ($\rho = -0.123$). 1.1 illustrates the relationship between PPD and carotene, and suggests that with increased amounts of carotene there is an

apparent reduction or delaying of PPD onset. The exceptions can be explained by samples having higher than average dry matter contents.

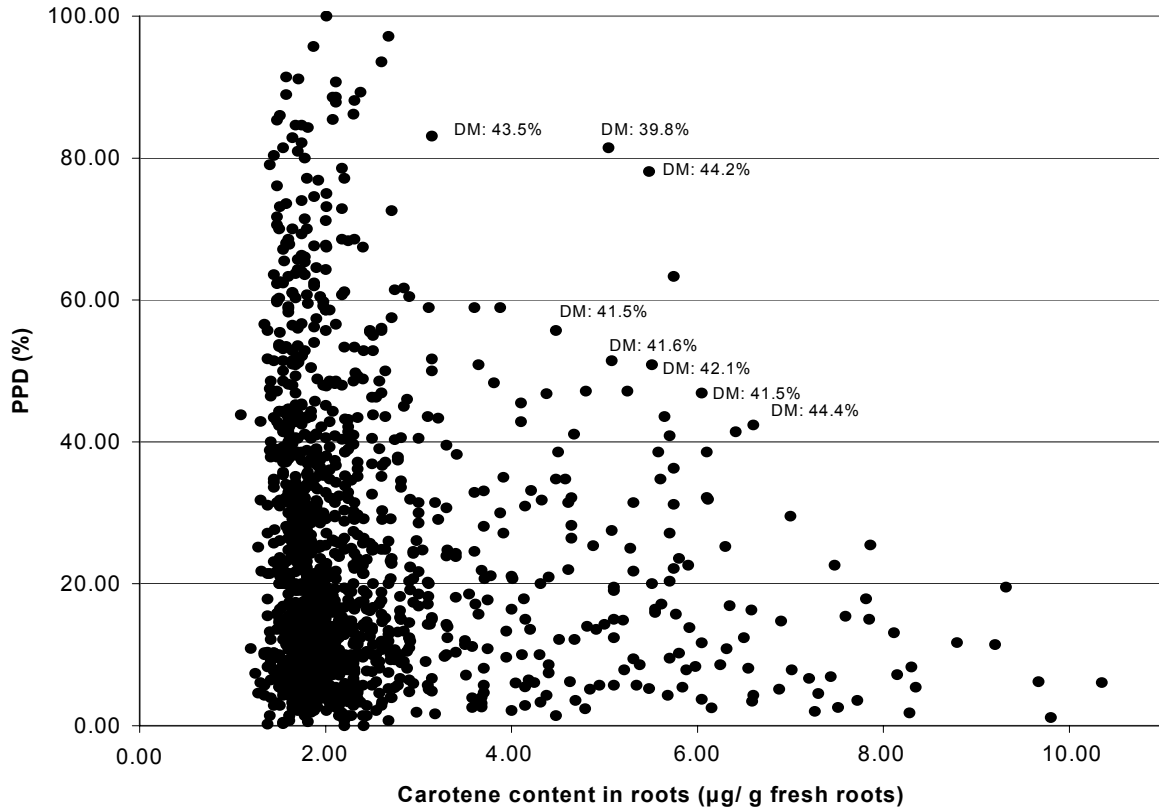


Figure 1.1. Relationship between carotene content ($\mu\text{g} / \text{g FT}$) and PPD (%) analyzed in a sample of 1315 cassava roots. Most data points in the upper periphery of the distribution came from root samples with dry matter content (%) considerably higher than the average for the sample analyzed.

Evaluation of PPD is prone to large experimental errors, because roots are left at room temperature (Wheatley et.al., 1985, Zapata, 2001) for seven days. Current measurements on PPD had to be carried out at different harvesting times, because of restriction in the availability of planting material from the germplasm bank and limitations in the number of clones that could be processed at any given time. Therefore, PPD estimates were probably affected by variations in the environmental conditions under which they were taken.

Other associations involving dry matter content.

Van Oirschot et al. (2000) reported a negative correlation between dry matter and sugar contents in cassava roots. This relationship was established on six cultivars and upon pre-harvest pruning of stems. In this paper, the association between these two variables is further confirmed but on a much larger sample (1374 clones) and with no pruning being involved.

Trace mineral concentrations.

All measurements were taken in mg kg⁻¹ or % and on a dry tissue basis. Because of their nutritional relevance, results from few elements were highlighted. Roots showed an average of 17.1 mg kg⁻¹ for iron, 7.5 mg kg⁻¹ for zinc, and 0.076% for calcium (Table 1.4). Significant but weak relationships between total carotene content and Mn, and Ca were found (correlation coefficients of 0.15 and 0.13, respectively). In general, the correlations between PPD and mineral concentrations in roots were low. The higher correlation coefficients found were negative: K ($\rho = -0.29$); S ($\rho = -0.24$); and N ($\rho = -0.21$). There seemed to be an inverse relationship between these minerals and PPD.

Table 1.4. Simple descriptive statistics (minimum, maximum, mean and standard deviation) for mineral and protein concentrations (dry weight basis) in roots from 600 cassava genotypes^a, and their correlations with carotene content and PPD.

Mineral	Minimum	Maximum	Average	St.Dev.	Correlations with	
					carotene	PPD
Element content measured in mg/kg						
Fe	6.0	230.0	17.1	15.2	0.09*	-0.12**
Mn	0.45	5.0	1.4	0.6	0.15**	-0.19**
B	1.14	9.91	2.0	0.6	0.07 ^{NS}	-0.14**
Cu	0.79	40.31	5.8	5.4	-0.02 ^{NS}	-0.06 ^{NS}
Zn	2.63	37.52	7.5	3.6	-0.07 ^{NS}	-0.03 ^{NS}
Na	18.6	1230.0	129.2	147.3	-0.02 ^{NS}	-0.04 ^{NS}
Al	4.4	330	11.5	20.4	na	na
Element content measured in %						
Ca	0.031	0.250	0.076	0.032	0.13**	0.06 ^{NS}
Mg	0.052	0.240	0.105	0.028	0.02 ^{NS}	0.00 ^{NS}
K	0.410	2p .500	1.172	0.321	-0.02 ^{NS}	-0.29**
P	0.071	0.320	0.165	0.036	-0.08*	-0.10*
S	0.012	0.055	0.027	0.008	0.04 ^{NS}	-0.24**
Crude protein content (%) ^b						
	0.769	8.313	3.063	1.418	0.02	-0.21**

^a Sample sizes for root evaluation of B = 580; for Cu = 599, and for Al = 460.

*, ** Significant at P < 0.05 and P < 0.01 probability level respectively. ^{NS}, non-significant.

na, Non-available

^b Correlations with carotene content and PPD estimated using N content.

Protein contents.

Table 1.4 also presents the estimates of crude protein content in root tissue. Averages for roots are slightly higher than those reported in the literature (Buitrago, 1990). A weak positive correlation ($\rho=0.14$) was also observed between nitrogen and HCN contents in the roots. Table 1.5 lists the best 30 clones regarding protein content in the roots. A high frequency of these clones come from Meso America.

1.1.c Discussion

The results presented in this study are exploratory in nature. The correlations among different variables, because of the size of samples involved, are very useful in suggesting associations that can be exploited to facilitate cassava genetic improvement. Furthermore,

one of the main purposes of this study was to evaluate the nutritional properties of cassava both for human and animals. It was of particular interest to determine the potential of cassava to provide carotenes through the diet as a contributing factor for alleviating vitamin A deficiency in human populations.

Table 1.5. List of the best 33 clones regarding crude protein content in the roots from a sample of 600 cassava clones. CM and SM codes identify clones derived from CIAT's cassava breeding project. The remaining clones are from the germplasm bank collection.

Clone and protein (%)		Clone and protein (%)		Clone and protein (%)	
CM 5620-3	8.31	MCOL 2436	6.25	MBRA 101	5.94
SM 1406-1	8.13	MBRA 26	6.25	MCOL 219	5.94
MCOL 689B	7.75	MCR 136	6.13	MGUA 33	5.94
MCOL 1563	7.38	MGUA 9	6.13	CM 7310-1	5.88
MGUA 76	6.94	MGUA 91	6.06	MCOL 678	5.88
MCR 142	6.63	MMEX108	6.06	MMEX 95	5.81
CM 696-1	6.44	SM 629-6	6.00	MGUA 79	5.81
CM 3199-1	6.44	SM 673-1	6.00	MBRA 300	5.75
SM 734-5	6.44	MCOL 2532	6.00	MCOL 2459	5.75
MCR 38	6.31	MGUA 19	6.00	MBRA 1384	5.75
MGUA 86	6.31	CM 3236-3	5.94	MCOL 2694	5.75

The lack of replication for the large number of genotypes screened is a strong limitation in this study. However, the variation associated with the experimental error of carotene concentration in the roots, has been measured (CIAT, 1999). Standard deviations for measurements of roots from different plants of the same clone, of different roots from the same plant and of different samples from the same root represented 7.7, 7.0 and 2.8% of the mean carotene concentrations, respectively. Carotene content is a stable trait and genetic differences remain relatively constant even when clones are grown in different locations as indicated by a preliminary study to measure the importance of genotype by environment interaction (CIAT, 2002). Likewise, estimates of the experimental errors associated with crude protein estimations are available. Crude protein content has been measured in roots from 132 clones in repeated occasions (from 2 to 4 measurements in the same genotype) always in different years and often using a different biochemistry laboratory. Mean protein content was 3.561 and the average standard deviation in these measurements was 0.282. Coefficient of variability for crude protein content in cassava roots was low (8.72%). Observed differences in crude protein content from the sample of 600 genotypes reported here, therefore, are expected to be largely genetic in nature. CIAT is currently growing a set of contrasting clones to measure the stability of Fe and Zn measurements and the relative importance of genotype by environment for these two traits.

Jalal et al, (1998) demonstrated that carotene rich, orange fleshed sweet potato (*Ipomoea batatas* (L) Poir) supplied for three weeks to a group of human individuals in Indonesia, resulted in changes in blood serum amount of retinol. The analysis of different mineral elements was also relevant, particularly in light of the emerging evidence of a synergistic effect between carotene, Fe and Zn. García-Casal et al. (1998) demonstrated that vitamin A and beta-carotene increased iron absorption from different sources. Also vitamin A has been

shown to contribute increasing hemoglobin content (a typical symptom of Fe-deficiency) as reported by Kolsteren et al.(1999) and Mwanri et al. (2000).

Results observed in the large samples analyzed demonstrate that cassava roots are a valuable source of carotene, which can help alleviating chronic vitamin A deficiency in human populations suffering from it. Although the negative association between carotene content and PPD is still preliminary it is a relevant issue: if higher carotenes in the roots reduce or delay PPD, this would encourage farmers to grow cassava clones with yellow roots, therefore helping to overcome the frequent reluctance by subsistence farmers to adopt new varieties. Further studies, under better-controlled conditions for measuring PPD, however, are needed for corroborating the preliminary evidence already found and are already underway.

The range of variation observed in the 1789 measurements was narrower than that reported by Iglesias et al. (1997). The highest value observed in the current analysis was 10.40 $\mu\text{g} / \text{g}$ FT, whereas in the previous report as much as 25.5 $\mu\text{g} / \text{g}$ FT have been reported for MBRA 516. In the current analysis, carotene content in the clone MBRA 516 was measured in two different opportunities providing values of 7.8 and 8.3 $\mu\text{g} / \text{g}$ FT. After Iglesias et al. (1997) publication, which was a preliminary report, carotene quantification was changed to be based on the spectrophotometry because of problems with the HPLC protocol employed that had become evident through time. The current results, therefore, are more consistent and reliable than those of Iglesias et al. (1997).

Regarding protein content in the roots (estimated through N measurements), the mean crude content of 3.06 % agrees with those reported in the literature. However, the few clones with high protein content (ranging from 5.75 to 8.31%) are remarkable. New root samples from the same clones will be evaluated again to confirm current expectations, and to have a better estimation of the effect of genotype by environment interaction in the expression of this trait. The weak correlation between nitrogen content and cyanogenic potential would suggest that a fraction of the nitrogen detected originated in the cyanogenic glucosides. This association, if confirmed, seems to be low enough to allow for the possibility of developing clones with high protein and low HCN in their roots.

A remarkable feature regarding protein content in the roots is that 12 out of the best 30 clones originated in Meso-America: Costa Rica, Guatemala and Mexico (Table 1.5). This proportion (40 %) is much higher than that of clones representing this region (6.3%) in the total sample of 600 clones. This would suggest that a genetic introgression from Meso-American, non-cultivated *Manihot* species might have occurred, resulting in a high frequency of cassava clones with increased protein content in their roots. About a dozen *Manihot* species grow wild in Meso- America (mainly *M. aesculifolia*, *M. gualanensis*, *M. isoloba*, *M. pringlei*, and *M. oaxacana*), and can readily cross with *M. esculenta* (Brücher, 1989). Distinctive characteristics of cassava clones from this region (particularly Guatemala) have been reported using simple sequence repeat markers (CIAT, 2001). Cassava clones from this region are currently recovered from the in vitro collection and will be carefully analyzed for their protein content in the roots in November 2004. If only a few of these clones did reproduce the high concentrations (above 5%) reported in this study, it would already be a major finding in cassava research with enormous potential in Asia, Africa and Latin America.

Activity 1.2. Variation in crude protein content in cassava (*Manihot esculenta* Crantz) roots.

1.2.a Materials and methods

The preliminary results described in Activity 1.1 suggested a good potential for genetic variability of protein content in cassava roots. Therefore, an extensive data search was conducted to identify cassava germplasm whose roots had been analyzed for protein content more than once. A total of 133 clones from the germplasm collection at CIAT, as well as a few improved clones (1.6), whose roots had been analyzed more than once were found. For each clone, the analysis was conducted in different years and on different roots from plants grown in different environmental conditions. One genotype had root samples analyzed four times. Seven clones had three quantifications on crude protein content and the remaining 125 clones had two different evaluations for protein content in their roots.

Dates reported in this study started to be generated in 1991, when roots from 125 clones were analyzed. In 1993 a second group of roots from 107 clones that had been screened in 1991 were evaluated again. The process continued with another sampling in 1995 (roots from 13 clones); 1999 (roots from 21 clones); and years 2000, 2001 and 2004 with three additional clones each year.

Table 1.6. Origin of the different accessions evaluated for protein content in root parenchyma

	# clones	Average	St. Deviation	Min	Max
Nicaragua	1	4.76	0.00	4.76	4.76
Colombia	31	4.10	1.39	1.43	7.56
Argentina	4	4.07	1.31	2.71	5.85
Guatemala	6	4.02	0.70	3.19	5.01
Brazil	16	3.94	1.38	1.95	6.73
Ecuador	8	3.82	1.34	2.36	5.83
Fiji	2	3.69	0.30	3.48	3.91
USA	1	3.67	0.00	3.67	3.67
Dom. Republic	2	3.59	0.67	3.11	4.06
Peru	17	3.51	1.48	1.39	7.26
Malaysia	2	3.41	0.11	3.34	3.49
Indonesia	1	3.27	0.00	3.27	3.27
Paraguay	10	3.19	0.74	2.20	4.95
Costa Rica	3	3.13	0.88	2.13	3.78
Philippines	1	2.91	0.00	2.91	2.91
Cuba	9	2.76	0.94	1.79	4.95
Venezuela	12	2.69	0.59	1.74	3.70
Thailand	3	2.67	1.42	1.12	3.91
Improved	4	2.61	0.95	1.85	3.95
Across study	133	3.46	0.75[†]	1.12	7.56

[†]Average standard deviation for the different origins.

Two plants per clone were harvested and the roots from the two plants combined. From all the roots harvested from a given clone 4-5 roots were randomly selected. Selected roots were peeled and washed. From the proximal, central and distal sections of each root a slice was taken. Samples from each root were mixed together and chopped into small pieces. Resulting chips were properly mixed to obtain a uniform sample of root tissue from the 4-5 original

selected roots. A 100 g sample was then taken and dried in an oven with forced ventilation at 60 °C for 24 hours. Dried samples were then grinded in a mill with stainless steel grinding tool. All sample processing was carried out to avoid as much as possible contamination from soil or any other source.

All solid samples were analyzed on an oven-dried basis. In the year 2000, root samples were sent to the Analytical Laboratory of University of Adelaide where the samples were analyzed using the total combustion gas chromatograph or Dumas method (Colombo and Giazzi, 1982). A Carlo Erba Instrument (model is NA 1500 series 2 Total Combustion Gas Chromatograph) was used in the quantification on 10-15 milligrams of samples. The limit of determination for the sample is calculated as 10 X the standard deviation of the blank.

All the remaining samples were analyzed at the plant tissue analytical laboratory at CIAT. Nitrogen determination was based on a modification of the Kjeldahl method (Skalar, 1995). The root samples were digested with a mixture of sulphuric acid, selenium and salicylic acid. The salicylic acid forms a compound with the nitrates present to prevent losses of nitrate nitrogen. The digestion of the samples started with hydrogen peroxide and with this step the larger part of the organic matter is oxidized. After decomposition of the excess of H₂O₂, the digestion is completed by concentrated sulphuric acid at elevated temperature (330 °C) with selenium as catalyst (Walinga et al. 1989; Novozamsky et al. 1983). Nitrogen was quantified colorimetrically on a Segmented Flow Analyzer. In the coloring process, salicylate, nitroprusside (catalyst) and active chlorine are added to form a green colored complex with the ammonium ion. The absorption was measured at 660 nm (Krom, 1980; Searle, 1984).

Protein content was estimated by multiplying N concentrations by a constant of 6.25, although Hock-Hin & Van-Den reported in 1996 that in the case of cassava this figure is probably ranging from 4.75 to 5.87. The original conversion factor has been maintained to facilitate the comparisons with previous reports. N quantification was based on dried root flour. Therefore, HCN had already been released before the quantification and no nitrogen (or trace amounts) from cyanogenic compounds should have remained.

Statistical analysis was made using the square root transformation because the original data ranged from 1 to 8% (Gomez and Gomez, 1984). Because roots from one clone were measured in four different occasions, roots from seven clones in three occasions and the remaining 125 clones were evaluated only once, the harmonic mean (2.04), rather than the actual average was used in the estimation of a common standard error for the comparison of the means of two different clones.

1.2.b Results.

Table 1.6 describes the origin of the 133 clones evaluated and main statistical parameters for clones grouped depending on their origin. The samples of clones evaluated should not be considered as representative of each country within the accessions included in the cassava germplasm collection at CIAT. Perhaps the only relevant information about the grouping of clones made for Table 1.6 is the remarkably low levels of proteins in improved cassava germplasm. The important information from Table 1.6 is the range of variation for protein content in the germplasm evaluated. The highest level was observed in an accession from Colombia with 7.56 % of crude protein. The lowest level was observed in a Thai variety with only 1.12% crude protein.

The analysis of variance for the variation in protein content ($\sqrt{\%}$) is presented in Table 1.7. The data analyzed did not come from a study designed for this purpose. It is the consolidation of information taken over the years and includes only data on protein content from roots of cassava clones that had been evaluated more than once. Data analyzed was based on the square root transformation. Results from Table 1.7 provide a strong evidence to support the hypothesis of a genetic variation in protein content in cassava roots. In other words the variation ranging from 1.12 to 7.54% crude protein to a considerable extent has a genetic origin. The relative magnitude of the error (variation from sample to sample from roots of the same clone) was small as well as the coefficient of variation, which was only of 5.56% (Gomez and Gomez, 1985; Steel and Torrie 1960),

Table 1.7. Analysis of variance for protein content in roots from 133 cassava clones. Data has been transformed by the $\sqrt{\%}$ protein) function

Source of Variation	df	Sum of Squares	Mean Squares	Test-f Probability
Clones	132	29.170	0.221	0.0000
Error	142	1.515	0.011	
Total	274	30.685		
Overall mean= 1.859 CV= 5.56%				

The total variation among the 133 clones evaluated is illustrated in Figure 1.2. The standard error for the difference between two means is also provided in this Figure. As expected the differences among means and the size of the standard error clearly suggest statistical differences among the germplasm evaluated.

1.2.c Discussion

Relatively little efforts have been made to learn about variation in nutritional quality of cassava roots. In general, it is considered that the only contribution of cassava roots is in term of energy from the starch present in them. This article provides information that demonstrates that cassava roots can also be a valuable (or at least a better) source of proteins. Chavez et al. (2004) provided additional information on the nutritional quality of cassava roots in terms of carotene, Fe and Zn contents.

Results from this study are indeed very promising. It should be highlighted that results from only 133 clones have been analyzed and that the world cassava germplasm collection has more than 6000 accessions. Therefore, it is valid to assume that there are excellent possibilities of finding cassava clones with roots with as much as 8% (or above) protein content. The possibilities of further increasing the natural range of variation for protein content through traditional recurrent selection methods are also very encouraging. This activity will come to support on going research to increase protein content in cassava roots through interspecific crosses (CIAT, 2003)

Based on the promising results reported in this article a group of “high-protein clones” have been planted and the protein content in the roots will be analyzed along as the genotype by environment interaction assessed to understand the relative importance of inheritance and

environment in the expression of protein content in the roots. Amino acid profiling of the protein in these roots will also be attempted.

It was surprising to observe the poor performance (for protein content) of the four improved clones included in this study. Although this is not conclusive evidence, these results would suggest that improving cassava for higher productivity might result in a gradual loss of high protein content originally present in the landraces, unless proper efforts are made to quantify and use protein content as criterion in the selection process.

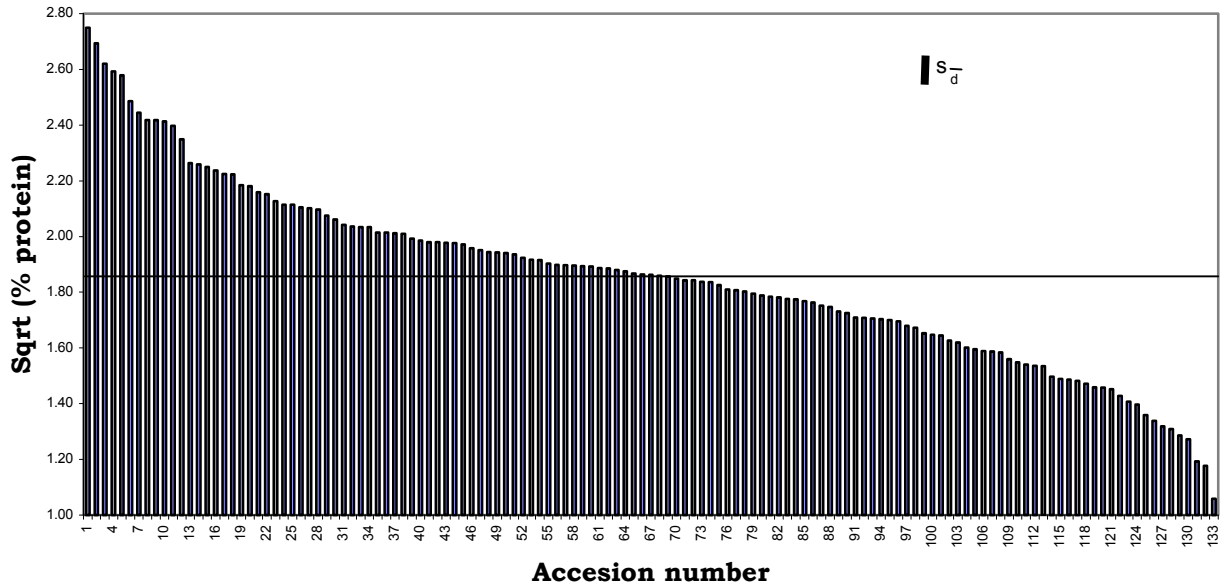


Figure 1.2. Variation in protein content (values transformed by the $\sqrt{(\% \text{ protein})}$ function) in roots from 133 cassava accessions evaluated. The standard error for the difference between means is included in the plot. The horizontal line shows the average protein content across the entire set of clones evaluated.

Activity 1.3. Reduction or delay of post-harvest physiological deterioration in high-carotene cassava roots.

1.3.a Materials and methods

Roots from a total of 101 cassava clones have been evaluated and a description of the origins of this germplasm is provided in Table 1.8. There were two types of clones, those produced from breeding projects at International Center for Tropical Agriculture (CIAT, Colombia) and clones from landraces from the germplasm collection held at CIAT.

Carotene concentration.

Randomly selected roots from plants of each clone were harvested after 11 months of planting and the carotene analysis was done immediately after harvest.

The extraction procedure outlined by Safo-Katanga et al. (1984) was modified including several extractions with acetone and petroleum ether (35-65 °C). Two or three commercially sized fresh cassava roots were peeled and cut into small pieces. The pieces were grated and blended using a household food processor. Approximately 5 g of tissue was obtained for extraction. Carotenoids extract was obtained by homogenization using a polytron homogenizer, followed by centrifugation to separate the liquid extract from the solid residue. The quantification was done by visible spectrophotometry using a Beckman DU 640 recording spectrophotometer. Detection was done at $\lambda = 450\text{nm}$ (Rodriguez Amaya 1989; 1990, 2001; Scott & Hart, 1993).

Table 1.8. Summary of the origin of the 101 cassava clones included in the present study.

Colombia	31	Costa Rica	2	Panama	1
Brazil	27	Indonesia	2	Thailand	1
CIAT - FS ^a	15	Malaysia	2	Venezuela	1
CIAT-HS ^a	6	Ecuador	1	TOTAL	101
Peru	6	Guatemala	1		
Argentina	4	Mexico	1		

^a CIAT-FS and CIAT-HS refers to improved clones derived from full-sib or half-sib families, respectively.

Post-Harvest Physiological Deterioration (PPD).

Five commercially sized roots (minimum length 18 cm) were randomly chosen. Roots were analyzed using the method of Wheatley et.al. (1985), with one modification: prepared roots were stored under ambient conditions for 7 days instead of 3 days. The proximal and distal root ends were cut off and the distal end were covered with clingfilm. After one week, seven transversal slices, 2 cm thick were cut along the root, starting from the proximal end. A score of 1-10 was assigned to each slice, corresponding to the percentage of the cut surface showing discoloration (1=10%, 2=20%, etc). The mean score of PPD for each root was calculated by averaging the score across the seven slices.

Because of the limitations in the number of roots whose carotene content could be determined each day, harvest took place from April 12 to April 28, 2004. Each day a sample of roots was taken for carotene quantification and another sample was used for PPD determination. To avoid variations in the environmental conditions in which the roots were maintained for PPD quantification, the roots were kept in a controlled environment chamber at 25 °C and 60-80% moisture.

Table 1.9. Descriptive statistics for the relevant traits evaluated in root from a sample of 101 cassava clones.

	Color intensity	Carotene content	Post-harvest physiological deterioration	Dry matter content
	(1-9)	$\mu\text{g} / \text{g FT}$	(%)	(%)
Average	3.13	2.06	20.08	34.52
St. Deviation	1.43	1.79	20.41	5.02
Maximum	8.00	7.74	73.14	45.05
Minimum	1.00	0.20	0.00	16.89

Dry matter content.

Dry matter content was estimated by weighting 20-30 g of chopped fresh roots and, then, drying them in an oven at 60 °C for 24 h. The relationship between dry and fresh weights (expressed as percentage) was used for estimating dry matter contents.

Root coloration and other measurements.

A 1 to 9 scale for the visual estimation of root coloration was developed and printed for a uniform estimation of color intensity. The color of root parenchyma can vary from white, cream, yellow, and orange. Pinkish roots (score 9) have also been observed in cassava.

Statistical analysis

The relationship between variables was evaluated through regression analysis. Different alternative models were considered for each case and the best one selected to be presented. In the case of PPD, a variable measured as percentage which presented many cases of values between 0-10 %, data was transformed using the Arcsin $\sqrt{\text{percentage}}$ transformation (Steel and Torrie, 1960). Dry matter content is also expressed as percentage. However, no data below 17% (only one data point) was found and, therefore, the Arcsin $\sqrt{\text{percentage}}$ transformation was considered not necessary.

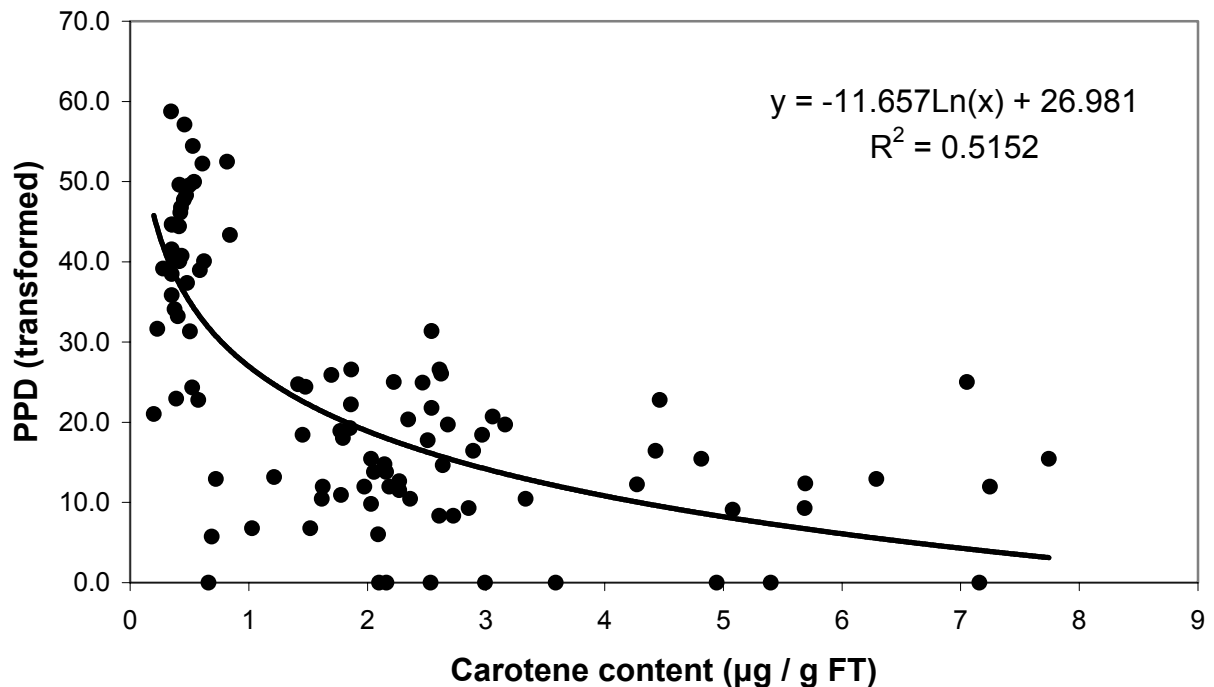


Figure 1.3. Relationship between carotene content and post-harvest physiological deterioration in roots from a sample of 101 cassava clones. PPD data has been transformed with the Arcsin $\sqrt{\text{percentage}}$ transformation

1.3.b Results.

Table 1.9 presents the descriptive statistical parameters for color intensity, carotene content, PPD and dry matter content from the roots of the 101 clones evaluated. The range of variation (from 1.79 to 7.74 µg / g FT) and average (2.06 µg / g FT) for carotene content

agree with those reported earlier (Chávez et al., 2004). Average PPD (20.08) and dry matter content (34.52%) also agree with previous results (Chávez et al. 2004; Cortés et al. 2002).

Figure 1.3 depicts the general relationship between post-harvest physiological deterioration and carotene content in the roots. As expected, roots with low carotene content (white parenchyma) were more frequent resulting in a clear asymmetrical distribution for carotene in the roots, which concentrated frequencies in the lower values to the left of the plot, and a long tale to the right (skewness value = 1.34).

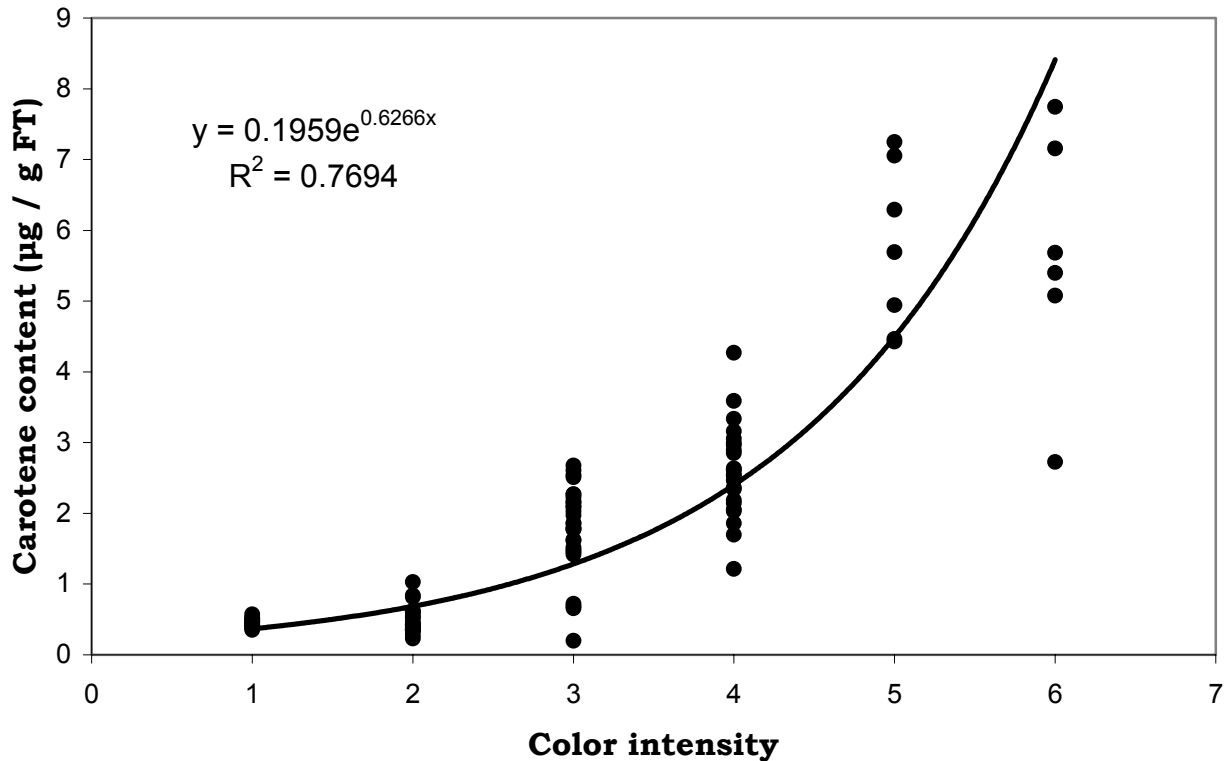


Figure 1.4. Relationship between carotene content and color intensity in roots from a sample of 101 cassava clones.

PPD values varied considerably (from 0.0 to 73.1%) at carotene contents lower than 1.0 µg / g FT, but then tended to have a ceiling (27.1%) at higher carotene contents (Figure 1.3). Results from Figure 1.3 suggest that other factors may play a role in determining PPD when carotene contents are lower than the 1.0 µg / g FT threshold. Regression analysis resulted in the equation $PPD = 26.981 - 11.657 \ln(\text{carotene content})$, with a coefficient of determination $R^2 = 0.515$. These results clearly indicate that PPD is negatively associated with carotene content (regression coefficient significantly different from zero with $P < 0.0001$) and that a significant proportion of the variability measured for PPD could be explained by the independent variable (carotene content).

Figure 1.4 illustrates the relationship between carotene content and color intensity in the root parenchyma. In this analysis one data point (color score = 8; carotene content 4.81 $\mu\text{g} / \text{g FT}$) was clearly outlying and was not considered in the analysis. The regression analysis suggested an exponential relationship (carotene content = $0.196 e^{0.627(\text{color intensity})}$), with a coefficient of determination $R^2 = 0.769$.

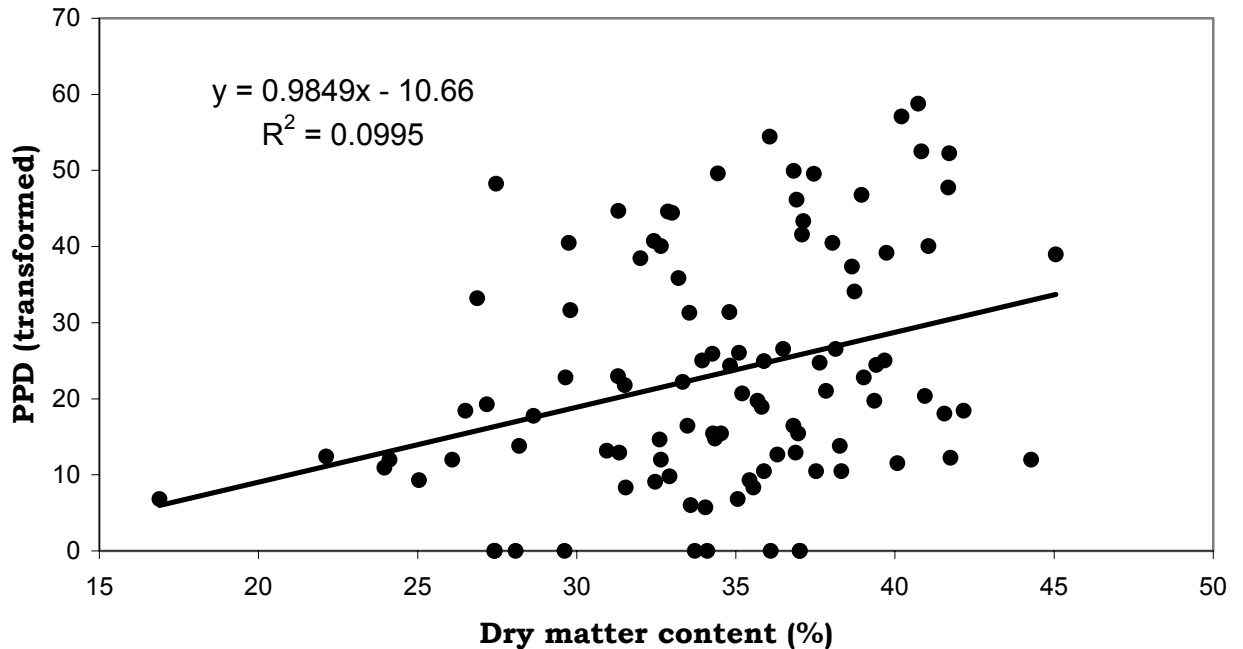


Figure 1.5. Relationship between post-harvest physiological deterioration and dry matter content in roots from a sample of 101 cassava clones. PPD data has been transformed with the Arcsin $\sqrt{\text{percentage}}$ transformation

Finally, the relationship between post-harvest physiological deterioration and dry matter content in roots is illustrated in Figure 1.5. This relationship is not as clear as the previous ones with a linear regression $\text{PPD} = -10.66 + 0.985 (\text{DMC})$, which resulted in a coefficient of determination $R^2 = 0.100$. This relationship is clearly a weak one.

1.3.c Discussion

Our results agree with those from van Oirschot, et al. (2000) who reported that PPD is positively correlated with dry matter content in the roots. This is an unfortunate situation because an important objective in cassava breeding projects is to increase root dry matter content, which in turn, will aggravate the PPD problem. However, in this study the relationship was a weak one with a small R^2 value. The most significant findings in the current study are the excellent association of carotene content in the roots with reduced or delayed PPD ($R^2 = 0.515$) and increased color intensity ($R^2 = 0.769$).

The negative association between carotene content and PPD is very promising because it suggests that yellow, high-carotene cassava roots are not only more nutritious, but also would have a better marketability because their reduced or delayed PPD. It should be mentioned, however, that high-carotene roots could have an increased shelf life of just one or two additional days. The beneficial effect of carotenes, in other words, is far from enough for overcoming this serious problem for marketing cassava roots. On the other hand this beneficial effect should encourage farmers to grow yellow rooted cassava clones.

The relationship between carotene content in the roots and color intensity is also relevant. Adequate laboratory facilities for the quantification of carotene contents in many developing countries and, particularly, in those regions where cassava is an important crop, are missing. The association between color intensity and carotene content clearly indicates that a simple selection based on visual scoring of color intensity should be enough for an initial selection of clones with high carotene content in their roots. Because of the exponential nature of this association, efforts should be directed at improving the color chart on which the color intensity score was based, particularly from scores ranging from 4 to 8.

Activity 1.4. Effect of processing on carotenes present in cassava roots.

1.4.a Materials and methods

Two similar experiments were conducted for two consecutive years. The first one involved the evaluation of roots from four cassava clones. The second experiment was conducted on root samples from three different cassava clones. In both experiments, carotene contents were measured in fresh roots and after processing through different methodologies: boiling, oven-drying, sun-drying, Gari, shadow drying, and lyophilized (lyophilized results are reported only for the first experiment and shadow drying only for the second one).

Measurement of carotene concentration through the colorimetric method.

Randomly selected roots from plants of each clone were harvested after 11 months of planting and the carotene analysis was done immediately after harvest. The extraction procedure outlined by Safo-Katanga et al. (1984) was modified including several extractions with acetone and petroleum ether (35-65 °C). Two or three commercially sized fresh cassava roots were peeled and cut into small pieces. The pieces were grated and blended using a household food processor. Approximately 5 g of tissue was obtained for extraction. Carotenoids extract was obtained by homogenization using a polytron homogenizer, followed by centrifugation to separate the liquid extract from the solid residue. The quantification was done by visible spectrophotometry using a Beckman DU 640 recording spectrophotometer. Detection was done at $\lambda = 450\text{nm}$ (Rodriguez Amaya 1989; 1990, 2001; Scott & Hart, 1993).

Measurement of carotene concentration through the HPLC method.

From the reading performed with the spectrophotometric quantification of total carotenes, aliquots (15 ml) of petroleum extract were partially dried by rota-evaporation and completely dried by nitrogen flux. Immediately before injection, the dry extract was dissolved in 1 ml of HPLC grade acetone and filtrated through 0.22 μm PTFE syringe filter. Twenty microliters were injected in the HPLC system using a YMC-C30 Carotenoid S 5 μm (250 mm x 4.6mm, Waters) column. Separation was performed by a isocratic elution with a mobile phase of methanol:methyl-t-butylether, 85:15 v/v, during 90 minutes at 0.8 ml min⁻¹ and 23°C. β -

carotene was detected by monitoring absorption at 450 nm. Identification and quantification were performed by comparing retention times and visible spectra with a standard of β -carotene.

The processing methods employed were:

Boiling roots: Each root is split in two upon harvest. Half of it goes to boiling. The five half-roots are then mixed and a sample taken for measurement. This treatment takes place the same day the roots are harvested. The half of each root, which is not boiled, will be used for the rest of the treatments. Root samples will be mixed and then undergo the different processing methods.

Oven drying: Samples are dried at 60 °C for 24 hours. Roots are coarsely chopped to simulate what is done in the chipping and drying facilities.

Liophylization: Samples are placed at -20 °C for 36 hours under vacuum conditions. After 36 hours samples are already dry. They are ground and are ready for analysis. This information is important for storing and/or shipping root samples until quantification can be made.

Sun-drying: Roots are coarsely chopped and placed under the sun for 2-3 days (depending on environmental conditions). As can be seen in the chronological table, there is room for making the quantification of sun-dried roots on Wednesday or Thursday of each week. If it is necessary to do it in the Thursday, then the Gari evaluation (planned to be made on Thursdays) can be moved one day ahead.

Shadow-drying: The same kind of root samples used for sun-drying, will also be dried under the same conditions but not under direct sun light. Since drying may require a longer period the analyses of these samples will take place five days after harvest on Fridays.

Gari: During the seventh week the gari preparations from the three replications will be boiled. Therefore, we will have an estimation of further losses of carotenes upon boiling the gari. There will be no replication for these treatments but a trend analysis after 3, 4 and 5 week of storage of the gari.

1.4.b Results

Table 1.10 provides the analysis of variance for the first experiment and Table 1.11 the averages and retention values after different processing methods. Results are based on the colorimetric and the HPLC quantification procedures separately. In this case the results from the HPLC quantification involves all carotenoid pigments and, therefore, it is equivalent to the total carotene measurements detected by the colorimetric method.

The variety by processing method interaction was significant for the colorimetric and HPLC measurements (Table 1.10) as were the differences among processing methods. On average, the differences between the four varieties were not significant, although the average carotene contents when measured in the fresh roots were very different.

Based on the results from Table 1.11 the lyophylization recovered the highest levels of carotenes. However, this is not actually a processing method but rather an approach

included to develop alternative storage protocols. The work on carotenes in cassava roots is always affected by the fact that roots cannot be stored for a long period, and the carotenes themselves are very liable. Since only a limited number of samples can be analyzed per day there is an ultimately strong limitation on the number of varieties, replications and/or processing methods that can be analyzed at any given time. Lyophilization was among one of the alternatives evaluated for storing cassava root samples until they can be analyzed for carotene content.

Table 1.10. Analysis of variance for carotene retention (measured through the colorimetric and HPLC methods) from roots of four cassava clones after different processing methods (lyophilization, boiling, oven-drying, sun-drying and Gari preparation). Results based on four replications for each treatment/variety combination.

Source of variation	df	Mean squares	
		Colorimetric	HP
Varieties (V)	3	739.8	1203.9
Processing method (P)	4	1438.8	6817.6
V x P Interaction	12	11339.5	13618.1
Error	60	377.8	608.8
TOTAL	79	70.7	214.7

The actual processing method that retained the highest levels of carotenes was boiling the roots (Table 1.11) with retention levels around 56%. Oven-drying resulted in an average retention (combining data from the colorimetric and HPLC quantification methods) of about 29.8 %, followed by sun-drying (27.6%) and finally gari (16.6%). In general the colorimetric method resulted in higher readings that the HPLC method.

Table 1.11. Average carotene content and retention (measured through the colorimetric and HPLC methods) from roots of four cassava clones after different processing methods (lyophilization, boiling, oven-drying, sun-drying and Gari preparation). Results based on four replications for each treatment/variety combination.

	Fresh roots	Lyophilized	Boiled	Oven dried	Sun dried	Gari
Carotenes measured through the colorimetric method						
CM 9247-17	4.56	3.36	3.15	1.81	1.16	1.01
CM 9249-23	6.03	5.81	2.86	1.73	2.06	1.18
CM 9629-1	4.17	3.94	3.17	2.56	1.74	0.95
CM 9679-25	9.64	8.33	4.38	1.79	2.77	1.60
AVERAGE	6.10	5.36	3.39	1.97	1.93	1.18
% retention	100	87.86	55.57	32.36	31.69	19.42
Carotenes measured through the HPLC method						
CM 9247-17	2.26	1.69	1.59	0.83	0.58	0.50
CM 9249-23	4.50	3.37	2.45	0.94	1.14	0.55
CM 9629-1	1.70	2.17	1.62	1.43	0.61	0.43
CM 9679-25	7.15	5.35	3.05	1.06	1.34	0.68
AVERAGE	3.90	3.14	2.18	1.06	0.92	0.54
% retention	100	80.57	55.81	27.23	23.51	13.85

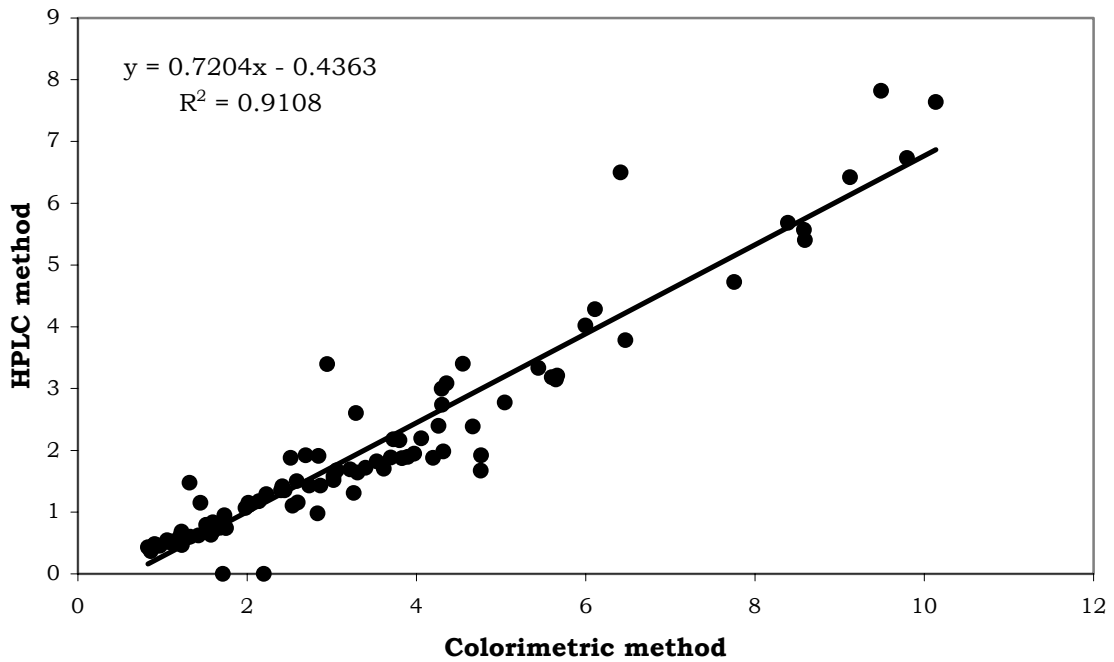


Figure 1.6. Relationship between carotene content (mg/100 g DW) measured in different samples of unprocessed or processed cassava tissue through the colorimetric and HPLC methods.

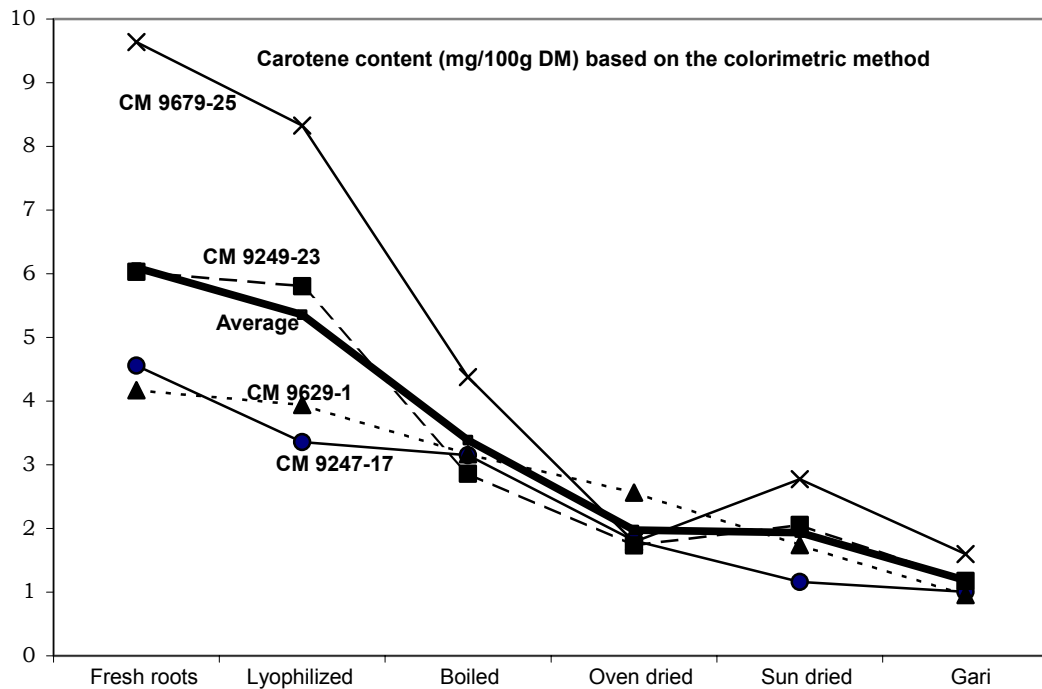


Figure 1.7. Effect of processing cassava roots from four different varieties on carotene content measured with the colorimetric method. Data from the first experiment.

One interesting result from this evaluation is the good correlation observed for colorimetric and HPLC measurements shown in Figure 1.6. Results from this plot suggest that the less expensive and faster colorimetric approach is precise enough to distinguish the roots or tissue samples with higher levels of carotenes. The data from the first experiment, summarized in Table 1.11, are also presented graphically in Figure 1.7.

Results from the second experiment are summarized in Table 1.12. The most important difference from Tables 1.11 and 1.12 is that in the later β -carotene, rather than total carotenes, were considered for the HPLC measurements. In general results are comparable to those observed in Table 1.11. Boiled roots retained the highest levels of carotenes (68.8% combined across the two measuring procedures), followed by oven-drying (60.6%), shadow-drying (47.9%); sun-drying (29.7%) and gari (27.8%). In general recovery levels in the second experiment were higher than equivalent ones in the first experiment. Another difference between the two experiments was that HPLC measurements for β -carotene were frequently higher than colorimetric measurements for total carotenes.

Table 1.12. Average carotene content and retention (colorimetric method) and average β -carotene and retention (HPLC method) from roots of three cassava clones after different processing methods (boiling, oven-drying, dark air, sun-drying and Gari preparation). Results based on three replications for each treatment/variety combination.

	Fresh roots	Boiled	Oven dried	Shadow dried	Sun dried	Gari
Total carotenes measured by the colorimetric method						
CM2772-3	11.06	2.94	5.00	4.64	3.03	2.78
MBRA1324	8.77	6.12	4.77	3.21	3.04	2.57
MCOL2401	21.05	21.32	9.56	7.20	4.25	3.55
Average	13.63	10.13	6.44	5.02	3.44	2.97
% retention	100.00	74.30	47.26	36.81	25.23	21.77
Total β-carotene measured by the HPLC method						
CM2772-3	10.47	1.69	6.57	6.43	3.33	3.67
MBRA1324	6.56	4.04	5.50	4.45	3.55	3.39
MCOL2401	13.07	13.34	10.16	6.90	3.44	3.14
Average	10.03	6.36	7.41	5.92	3.44	3.40
% retention	100.00	63.35	73.87	59.05	34.25	33.92

In Figure 1.8 the relationship between total carotenes measured through the colorimetric method and β -carotene measured through the HPLC approach is illustrated. It is clear that the colorimetric method is also appropriate for a quick assessment of the amount of β -carotene present in cassava roots and/or processed tissues from cassava roots. Figure 1.8 provides the regression between colorimetric measurements and β -carotene levels, as well as the r^2 values for that regression line (0.8725), which is quite satisfactory.

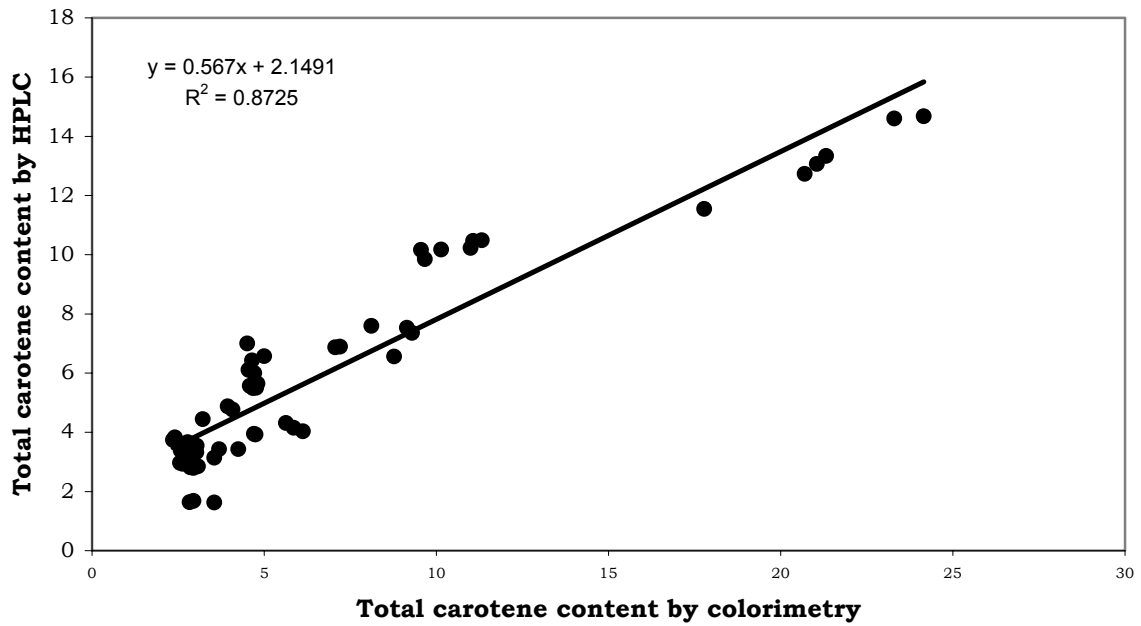


Figure 1.8. Relationship between total carotene content (colorimetric method) and total β -carotene (HPLC method) measured in different samples of unprocessed or processed cassava tissue.

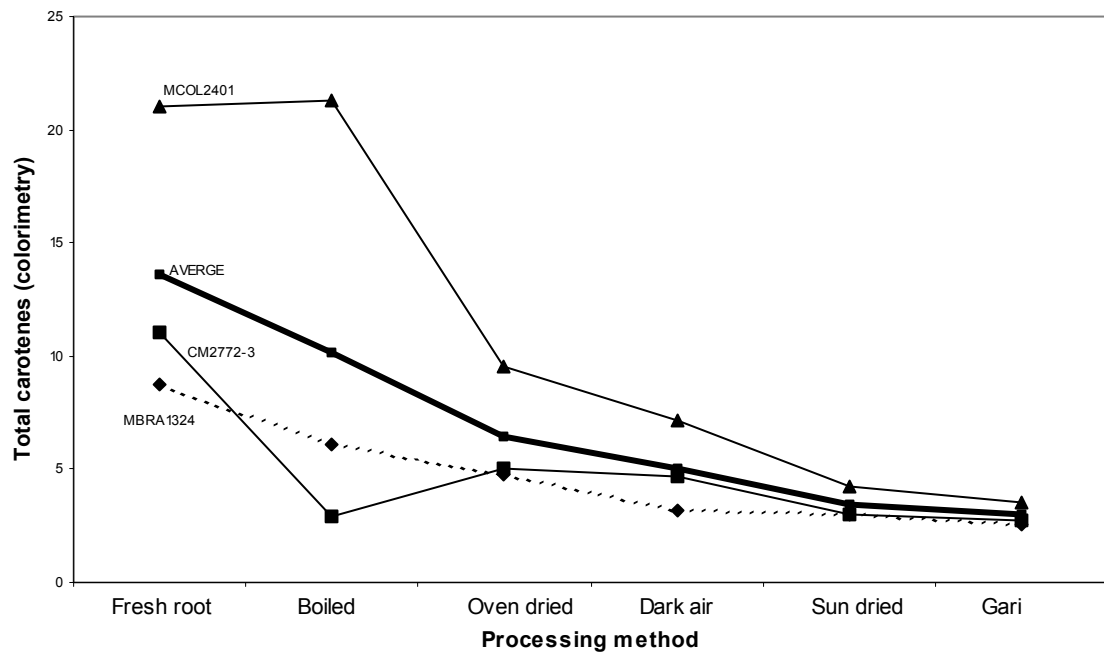


Figure 1.9. Effect of processing cassava roots from four different varieties on carotene content measured with the colorimetric method. Data from the second experiment.

Results from the second experiment showed unusual variation for boiled roots. CIAT will conduct further work for better understanding why in one case (MCOL2401) boiled roots contained more carotenes than fresh roots. One feasible explanation is that in some cases the boiling of the root tissue could release from the matrix higher levels of carotenes in one clone and much lower in other clones (i.e. MBRA 1324).

1.4.c Discussion

Results from this study are important for several reasons. The deployment of carotene-rich cassava cultivars depends on the proper selection of elite germplasm, including the capacity of identifying carotene-rich clones. Because of the difficulties in shipping and storing cassava roots selection for the high-carotene trait has to take place (at least for the time being) where the plants are grown. This implies that every country where Harvest Plus will conduct research with cassava needs to have the capacity of identifying high-carotene cultivars. Results from the different studies conducted in the last few years provide evidence that the selection of cassava clones whose roots have higher levels of carotenes can be conducted in a step-wise fashion. A first selection can be made through the visual scoring of color intensity, which as demonstrated by Activity 1.1, provides enough accuracy for sorting out high and low levels of carotenes. Roots selected for their high color intensity can be further screened by a more precise approach with the colorimetric protocol. This second selection will be able to more precisely detect those clones that, having approximately the same color intensity in their roots, vary in the actual levels of total carotenes. The colorimetric method allows as many as 40-50 evaluations per day, requires a relatively simple-equipment (the most sophisticated would be the spectrophotometer) and can be established or is already available in all the countries where HarvestPlus will implement research with cassava. Eventually it may be useful to get more precise information on the type of carotenes present in the few clones selected after the colorimeter phase. If that were the case, then roots could be analyzed through HPLC. In this case, because the number of clones and samples to analyze is considerably smaller, it is feasible to think about special deliveries of roots away from the place where the plants were grown.

Another important conclusion is that boiling roots provides the highest levels of recovery of carotenes. This information is very useful for determining regions where the bioavailability could be conducted. Those communities that consume cassava by boiling the roots are those more likely to benefit from carotenes present in the roots. Oven drying allowed the recovery of as much as 60% of the original levels of carotenes. This is also important because dried yellow roots could be a valuable source of pigments for the poultry feed industry, which could replace artificial drying products with natural pigments from cassava roots. If the industry becomes more interested in yellow roots, an obvious consequence is that there will be more yellow cassava roots in different communities and eventually more frequent consumption of this type of roots by humans.

Further information has also been obtained from the HPLC analysis of fresh cassava roots and after they were processed in the second experiment. This information relates to the proportion of different isomers of β -carotene (All trans β -carotene; 9-cis β -carotene; and 13-cis β -carotene). Results from this study suggests that about 60% of the β -carotene measured in fresh or processed cassava roots are trans β -carotene, the remaining 40% are equally distributed with 9-cis β -carotene; and 13-cis β -carotene. This information is important because it suggest that the relative proportion of different isomeric forms does not change substantially with different processing methods. Different isomers have different capacity to be turned into retinal (vitamin A).

Activity 1.5. Evaluation of different storage conditions of carotenes from fresh and processed cassava roots.

A major bottleneck for research on carotene content in cassava roots is their rapid deterioration, which prevents storage between harvest and analysis time. In addition, carotenes are liable and can easily degrade in the presence of light, for example. Therefore, further studies were conducted to evaluate alternative storage conditions that will allow delaying the analysis of carotenes without their degradation. In many ways this activity is related to **Activity 1.4**.

The experiment was conducted on root samples from three different cassava clones. Carotene contents were measured in fresh roots and after different storage conditions: storage at -20 °C; storage at -80 °C; lyophilized. Table 1.13 lists the main treatments evaluated through different cassava tissue, under different conditions, and at different time periods. In addition to these treatments the gari produced as described in Activity 1.4 was evaluated 3, 4 and 5 weeks after preparation. All treatments will be compared with the original levels and types of carotenes measured in fresh roots soon after harvest.

Table 1.13 Conditions evaluated for storing different cassava tissue por varying periods of time in relation to stability of carotene contents.

Storage conditions	Storage period (weeks)						
	2	4	8	12	16	20	24
Fresh roots							
Storage at -80 °C							
Storage at -20 °C							
Lyophilized							
Processed roots							
Oven dried							
Oven dried / vacuum sealed							
Sun dried							
Sun dried / vacuum sealed							

Measurement of carotene concentration through the colorimetric method.

Randomly selected roots from plants of each clone were harvested after 11 months of planting and the carotene analysis was done immediately after harvest. The extraction procedure outlined by Safo-Katanga et al. (1984) was modified including several extractions with acetone and petroleum ether (35-65 °C). Two or three commercially sized fresh cassava roots were peeled and cut into small pieces. The pieces were grated and blended using a household food processor. Approximately 5 g of tissue was obtained for extraction. Carotenoids extract was obtained by homogenization using a polytron homogenizer, followed by centrifugation to separate the liquid extract from the solid residue. The quantification was done by visible spectrophotometry using a Beckman DU 640 recording spectrophotometer. Detection was done at $\lambda = 450\text{nm}$ (Rodriguez Amaya 1989; 1990, 2001; Scott & Hart, 1993).

Measurement of arotene concentration through the HPLC method.

From the reading performed with the spectrophotometric quantification of total carotenes, aliquots (15 ml) of petroleum extract were partially dried by rota-evaporation and completely dried by nitrogen flux. Immediately before injection, the dry extract was dissolved in 1 ml of HPLC grade acetone and filtrated through 0.22 μm PTFE syringe filter. Twenty microliters were injected in the HPLC system using a YMC-C30 Carotenoid S 5 μm (250 mm x 4.6mm, Waters) column. Separation was performed by a isocratic elution with a mobile phase of methanol:methyl-t-butylether, 85:15 v/v, during 90 minutes at 0.8 ml min⁻¹ and 23°C. β -carotene was detected by monitoring absorption at 450 nm. Identification and quantification were performed by comparing retention times and visible spectra with a standard of β -carotene.

Harvest of the roots began in May and the last quantification of carotene contents (after 24 weeks) will take place during November, 2004. The evaluations evolved without major problem or unexpected difficulties. As soon as the last samples are measured the results of the chromatograms will be integrated for final analysis and publication in a refereed journal.

Since no data has been completely analyzed, no conclusion can be mentioned regarding the results. However, it should be emphasized that the information generated by this study will be very useful for facilitating the logistics of research of carotene content in cassava roots. For example, by the end of year 2004 a collection of intensely pigmented roots in the North Eastern State of Maranhão in Brazil has been planned. Carotene data from the many samples to be hopefully harvested was highly desirable. Otherwise an additional year would be required to grow the plants from the collected stakes. However, the conditions where collection is expected to take place do not allow for the proper and immediate analysis of root samples. Knowing about the effect of different storage conditions would greatly facilitate this work.

Activities 1.4 and 1.5 were technically supported by P. Nestel (Nutrition Coordinator, HarvestPlus) and D. Rodriguez Amaya (expert in carotene measurements at Universidad de São Paulo, Campinas, Brazil). Their contributions are herein acknowledged and thanked.

Activity 1.6. Measurement of genotype by environment effect on carotene, Fe and Zn contents in cassava roots.

As described in the article presented as Activity 1.1, interesting results were observed for Fe and Zn contents in cassava roots. These results would suggest that there is ample genetic variation for these traits and, therefore, hopes for developing and deploying high-Fe and or High-Zn cassava cultivars. One major problem of the data so far generated, however, is that they were based on single flour samples. This activity aims at measuring the relative importance of environment and genotype in the amount of Fe and Zn in cassava roots from different clones.

Crosses for genetic studies

A group of clones was selected because of their contrasting levels of Fe and Zn in their roots (Table 1.14). These clones will be used for further studies to measure the heritability of the trait, genotype by environment interaction, and eventually in breeding work. Below is the result of the most important characteristics of the four groups identified and selected. Carotenoid content was not considered as a selection criterion, but was measured in these

samples, nonetheless. These clones are currently recovered from the germplasm bank to produce planting material and are hardened in greenhouse conditions. By October 2004 the hardened seedlings will be moved to the screen-house and early 2005 will be transplanted to the field for evaluation. Harvest will take place during the second semester, 2005.

Table 1.14. Fe, Zn and carotene contents in roots from a group of cassava clones contrasting for these traits.

Fe and Zn contents	Number of clones	µg/100 fresh weight			Dry matter content (%)
		Carotene	Fe	Zn	
High-High	5	2.1	91.4	26.1	25.62
High-Low	5	2.6	64.8	5.1	35.93
Low-High	5	2.0	118	17.0	37.64
Low-Low	6	2.8	7.6	3.2	43.02
Overall	21	2.4	16.9	7.5	35.91

Measurement of the effects of environment of Fe and Zn accumulation in the roots.

The purpose of the study is to quantify the relative importance of GxE interactions in carotene, Fe and Zn contents in cassava roots. A group of ten clones were planted in four diverse environmental conditions, with three replications per location. Trials will be harvested in November 2004. Clones that have yellow roots (with certain variation in intensity) were included. Among the clones planted one has been found to contain higher than average Fe in its roots, and two lower than average Fe in their roots. It is important that soil samples are taken from each location as well, so soil samples will also be analyzed for their mineral contents.

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