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

The overall objective of this output is to improve the nutritional status of people living in marginal environments of the tropics, by selecting and promoting cassava genotypes with high and good bio-availability of micronutrients and vitamins. Related traits are the need for a better understanding of the biochemical and genetic basis of post-harvest physiological deterioration and starch quality traits.

Activity 1.1. Evaluation of genetic diversity for carotene content in cassava roots. Collaborative project with IFPRI.

Rationale.
This is one of the many collaborative activities between projects **SB2** and **IP3**. Most of the emphasis in relation to cassava breeding has been centered on increasing root production and concentration of starch. Since cassava is a staple in regions where there are severe deficiencies of micro-nutrients; the crop can be used as a vehicle to deliver vitamins and minerals in higher concentrations. Improving the efficiency with which cassava acquires micro-nutrients and accumulates them in the roots and leaves can have an enormous potential not only in terms of human nutrition, but also in terms of crop production.

In many respects, PPD resembles wound responses found in other better studied plant systems but cassava appears to lack the wound healing capacity which is normally associated with the inhibition of wounding responses. An important component of these wound responses are the oxidative processes. Carotene is known to have antioxidant properties. Therefore, PPD was measured in a sample of genotypes to evaluate the potential correlation between this pro-vitamin and PPD. A major problem regarding PPD is obtaining reliable data, because this trait is seriously affected by the environment, and handling of the roots. Repeatability is not as good as desirable, so some efforts have been made to reduce experimental error in the measurements.

In the field of human nutrition (and animal nutrition as well) there is an increasing amount of evidence of a synergistic effect between vitamins and certain minerals. It seems that iron and zinc contents in the diet increase vitamin A absorption and vice versa. Therefore, for the study of micro-nutrients availability from cassava roots and leaves, it is also important to measure mineral contents, because of the putative synergistic effects in their availability in the process of digestion.

Specific Objectives:
a) *To continue the screening of cassava elite clones and landraces from CIAT's germplasm collection for total carotene and minerals content in roots and leaves;*
b) *To correlate vitamin contents with physiological post-harvest deterioration.*
c) *To search for genotypes with higher protein content in the roots.*

Materials and methods

Harvesting and sampling. Root samples from 99 clones from the germplasm or the cassava-breeding project at CIAT were used for this study. Clones with colored roots were favored for the study. This represents a departure from the activities carried out previously, when white and colored roots were analyzed. The emphasis now focuses in identifying clones with the highest carotene content in the roots to be used in breeding and basic studies. Few white rooted clones were also included for the comparison of different methodologies for quantifying carotenes content. Harvest took place at around 10 months of age (normal harvesting time for cassava at CIAT) and commercial size, disease-free roots were taken to represent each clone.

*Carotene concentration measurements***.**

Colorimetric method. The extraction procedure outlined by Safo-Katanga et al. (1984), was modified by extracting root parenchyma with petroleum ether. The extraction protocol for leaves had to be modified due to the presence of tannins and chlorophylls. The modified protocol included several extractions with petroleum ether 35-65 ºC and washing steps with methanol in order to minimize the interference from the others pigments. A sample of 5 g was taken out of the root or leaves, randomly selected 10 to 11 months after planting. The quantification was done by ultraviolet spectrophotometry using a Shimadzu UV-VIS 160A recording spectrophotometer. UV detection was done at l = 455nm for root extracts and l = 490 nm for leaf extracts.

HPLC method. Starting from the method used for the colorimetric quantification of total carotenes, aliquots (20 ml) of petroleum extract were completely dried by rota-evaporation. Then the dry extract was dissolved in 1 ml of HPLC mobile phase (methanol: methyl-terbutyl-ether: water, 80:15:4 v/v), centrifuged at 14000 rpm and 10 µl were injected in the HPLC system using a YMC carotenoid 5 um, 250 mm x 4.6mm column. Separation was done by isocratic elution with a mixture of acetonitrile:methylene chloride: methanol, 70:20:10 v/v, as mobile phase at 1 ml min^{-1} and 30 °C. β-carotene was detected by monitoring absorption at 450 nm. Identification and quantification was done by comparing retention times and uv-vis spectra, with a standard of β-carotene (Sigma C-0126).

*Post-Harvest Physiological Deterioration (PPD) measurements***.** 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 7 days, 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.

Results

Analysis of total carotene content. Carotene concentration in the roots, based on the colorimetric method, ranged from 0.141 mg/100 g FW to 1.071 with a mean of 0.416 mg/100 g FW and a standard deviation of 0.240 (Table 1.1). These values are similar to those found in the group of more than 1000 accessions evaluated the previous years (see 2000 and 2001 Annual Reports).

Table 1.1 Evaluation of roots from 99 cassava clones for carotene content in the roots (mg/100 g FW) using to quantifying methodologies.

Parameter	Colorimetric method	HPLC method	
	Total carotenes	β-carotene	α-carotene
Average	0.416	0.521	0.039
Standard Deviation	0.240	0.402	0.042
Maximum	1.071	1.688	0.194
Minimum	0.141	0.077	0.000

Activity 1.2. Determination of the actual proportion of different carotenoids included in the total carotene measurement.

Rationale

In the measurement of total carotenes several chemical entities are considered. These entities vary greatly in their vitaminic activity (i.e. β-carotene being much more efficient than α–carotene). Because the ultimate interest is to evaluate the nutritional value of high-carotene cassava roots, it is necessary to get an approximation to the relationship between the "total carotene" variable and "vitaminic activity".

Total carotene is a relatively easy measurement to carry out. On the other hand, the determination of the different carotenoids requires HPLC procedures that are expensive and slow. The methodologies for measuring carotenes have been described above in **Activity 1.1**.

Objectives

a) To determine the proportion of different carotenoids making up the "total carotenes" measurement obtained in this output.

b) To produce preliminary data on how constant (across different genotypes) is the proportion of the different carotenoids making up the total carotene measurement.

Results

Comparison of the two methods for quantifying carotenes. The results of the two methodologies for measuring carotenes have been presented in Table 1.1. There is a striking difference between the results of the two methodologies. Adding up α- and β-carotenes estimated by the HPLC method yielded estimations about 34% larger than the total carotene quantification based on the colorimetric method (0.560 versus 0.416 mg/100 g FW). Moreover, the HPLC method allowed concluding that about 93% of the carotenes present in the roots is β-carotene. The relationship between total carotenes by colorimetry and β-carotene by the HPLC method is illustrated in Figure 1.1.

The ratio of α- over β-carotene was relatively uniform with an average of 0.07, ranging from non-detectable levels of α-carotene to a maximum of 0.19. One sample was not considered in this range because of its atypical value (a ratio of 0.43).

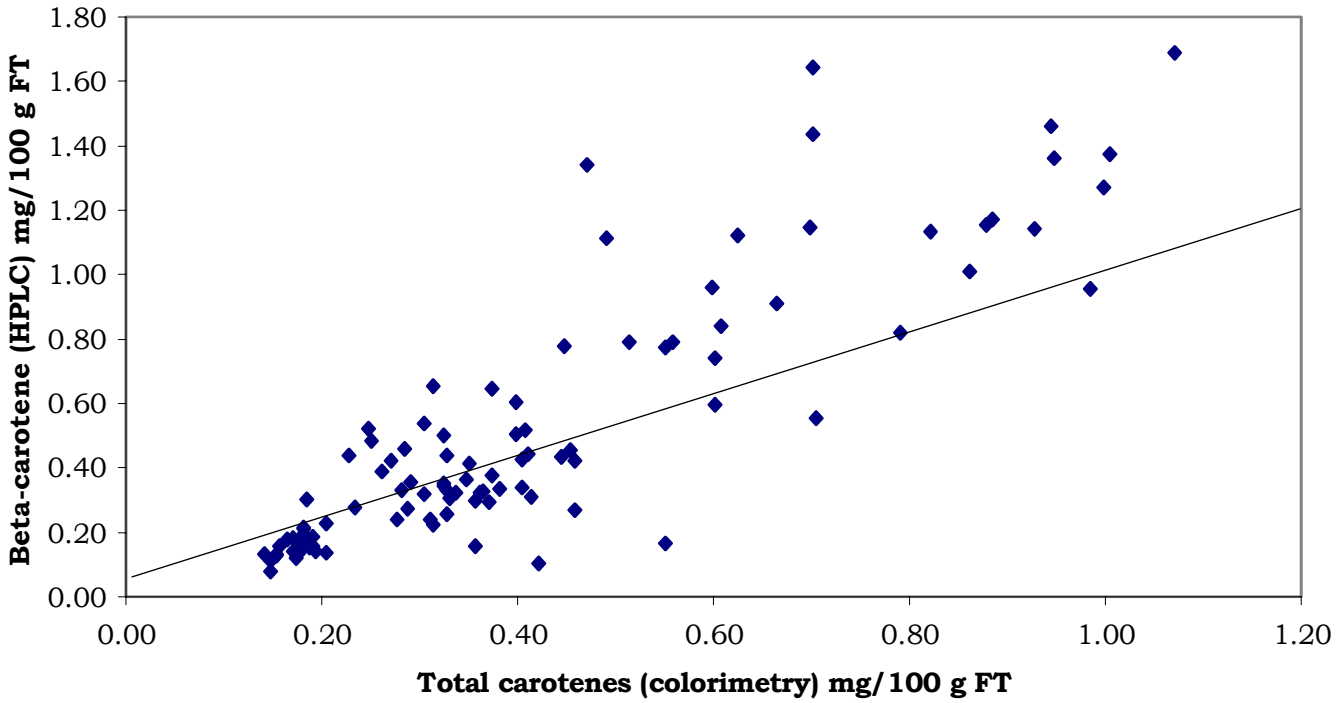

Figure 1.1. Relationship between total carotenes (measured by colorimetry) and β-carotene (measured by HPLC) in root samples from 99 cassava clones.

Activity 1.3. Further refinement of the association between post-harvest physiological deterioration with carotene content and other relevant traits in the root.

Rationale

One of the major constraints upon cassava production as a commercial crop is the rapid deterioration of its roots. Post-harvest physiological deterioration (**PPD**) often begins rapidly within 24 hours (Beeching et al., 1998). It consists of discoloration of the parenchyma as blue-black vascular streaks. Because of PPD, cassava roots need to be consumed shortly after harvesting (van Oirschot, et al., 2000). The short post-harvest storage life of cassava is a characteristic that limits the marketability of the roots. It implies high marketing margins and risks, as well as restricted management flexibility for farmers and processors.

Objectives

a) To further refine the relationship between PPD and carotene content and other relevant traits.

b) To improve the facilities where PPD measurements are taken, to reduce environmental effects.

Results

Data from previous years were consolidated and analyzed. A total of 1315 clones produced a very solid database. HCN does not seem to have a strong effect on PPD, whereas carotene did reduce and/or delay the onset of PPD (ρ = -0.123). Figure 1.2 illustrates the relationship between PPD and carotene, and suggests the occurrence of a threshold effect with carotene values lower that 50 mg having gradually lesser effect in reducing or delaying PPD. Sugar contents were negatively associated with PPD probably due to their negative correlation with dry matter content. Based on data presented (sample size=1315) the best regression model for PPD was:

PPD = -19.227 + 1.275 (Dry matter content) – 14.554 (Carotene)

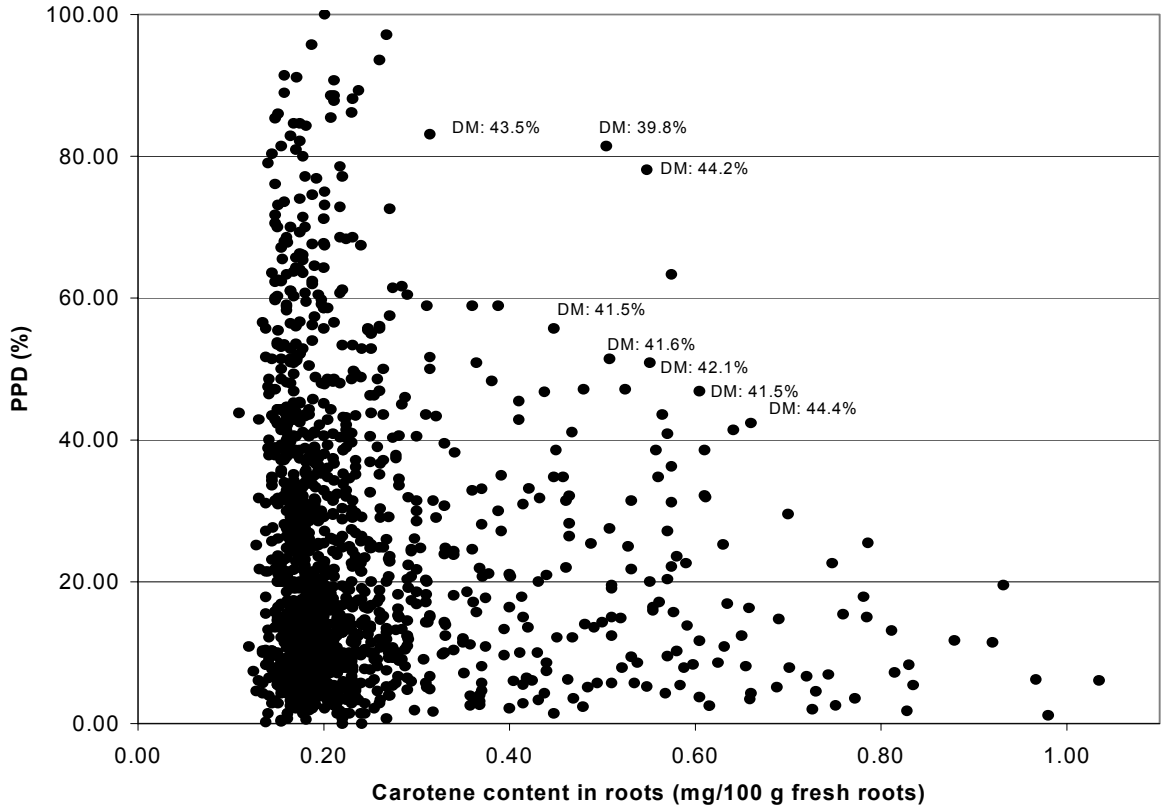

Figure 1.2 Relationship between carotene content (mg/100 g FT) and PPD (%) analyzed in a sample of 1315 cassava roots. Most data points in the upper periphery of the distribution came from root samples with dry matter content (%) considerably higher than the average for the sample analyzed.

In this model, the positive and negative associations of PPD with dry matter and carotene content, respectively, is further reinforced. The adjusted R^2 value for this model, however, was still low (0.132). The large amount of variation that remains unaccounted for is probably due to the large variations for PPD observed at low carotene contents values (Figure 1.2) or around the mean dry matter content. Also, evaluation of PPD is prone to large experimental

errors, because roots are left at room temperature (Wheatley et al., 1985) for seven days. Atmospheric changes in temperature and relative humidity are known to severely affect PPD development (Zapata, 2001). Current measurements on PPD had to be carried out at different harvesting times, because of restriction in the availability of materials 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.

A chamber with controlled temperature and, within certain ranges, relative humidity has been constructed with the support of CONGELAGRO-Mc Cain Foods. This chamber (Figure 1.3) is large enough to be able to evaluate the reaction to PPD of thousands of cassava roots simultaneously. Also since measurements are made under controlled conditions, the results will be comparable from batch to batch, in evaluations taken months apart from each other.

Figure 1.3. Photograph of the chamber with controlled temperature and relative humidity for more reliable and comparable measurements of PPD.

Activity 1.4. Further analyze other nutritional traits in cassava roots and leaves.

Rationale

Considerable amount of information has been produced in the previous analyses of cassava leaves and roots. The consolidated data provided a better base for relevant trends. Crude protein content in the roots was estimated (based on N measurements) in root samples of

600 cassava clones (CIAT, 2001) and further analyzed with other traits, as well as, looking at the geographical distributions (if any) associated with particular traits.

Objective

1) *To consolidate and further analyze data obtained in the last three years in search of useful information regarding the nutritional quality of cassava roots and/or leaves.*

Results

Regarding protein content in the roots (estimated through N measurements), the mean crude content of 3.06 % agrees with those reported in the literature. The few clones with high protein content (ranging from 5.75 to 8.31%) are remarkable. However, new root samples from the same clones will have to 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, however, was low enough to allow for the expectation of developing high protein-low HCN clones.

Table 1.2. 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. Highlighted are the clones from Central America.

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

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 1.2). 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. About a dozen *Manihot* species grow wild in Middle 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 Central America (particularly Guatemala) have been reported using simple sequence repeat markers (CIAT, 2001).

Activity 1.5. To estimate the relative importance of genotype by environment interactions in the expression of carotene content in cassava roots.

Rationale

Cassava is characterized by a notable genotype by environment interaction in many traits. Carotene content in the roots and leaf tissues is considered to be a relatively simple and stable trait, poorly affected by environmental factors. However, some preliminary data was required to confirm this assumption.

Objective

1) *To determine the relative importance of genotype by environment interaction in the expression of carotenes in the roots.*
2) *To measure the amount of carotenes remaining after different processing techniques by the HPLC quantifying method.*
3) *To determine the effect of freezing at –80 or –20 °C or upon lyophilization.*

Materials and methods

Four trials were conducted in four different locations in the Acid soil savannas. In the trials three contrasting cassava clones (for carotene content in the roots) were grown. Roots from each clone were harvested and delivered to CIAT-Palmira for carotene content analysis. The methodology used was the traditional colorimetric method described above.

Results

Very little genotype by environment interaction for carotene content in the roots was observed as illustrated in Figure 1.4. No relevant ranking cross-over was observed and the clone with the highest level of carotene content in the roots clearly maintained its superiority through the four environments evaluated. The variations among locations, with Santa Cruz showing the highest levels of carotene content and La Libertad and Cabuyal with the lowest can be explained by variations in dry matter content in the roots.

The results on stability of carotenes after different processing alternatives (boiling, sun drying and oven drying is underway. Preliminary results confirm that boiling the roots is the processing method with lowest loss of carotenes (about 75% of the original levels still present after boiling).

The last studies aim at establishing the stability of carotenes after a period of storage under three different conditions (at –80 or –20 °C or upon lyophilization). The purpose of this experiment is to determine alternatives for storing cassava roots for a certain period of time until their carotene content can be measured. Currently, all measurements are conducted with fresh roots, creating bottlenecks at harvest time, and limiting the number of samples that can be analyzed each growing cycle.

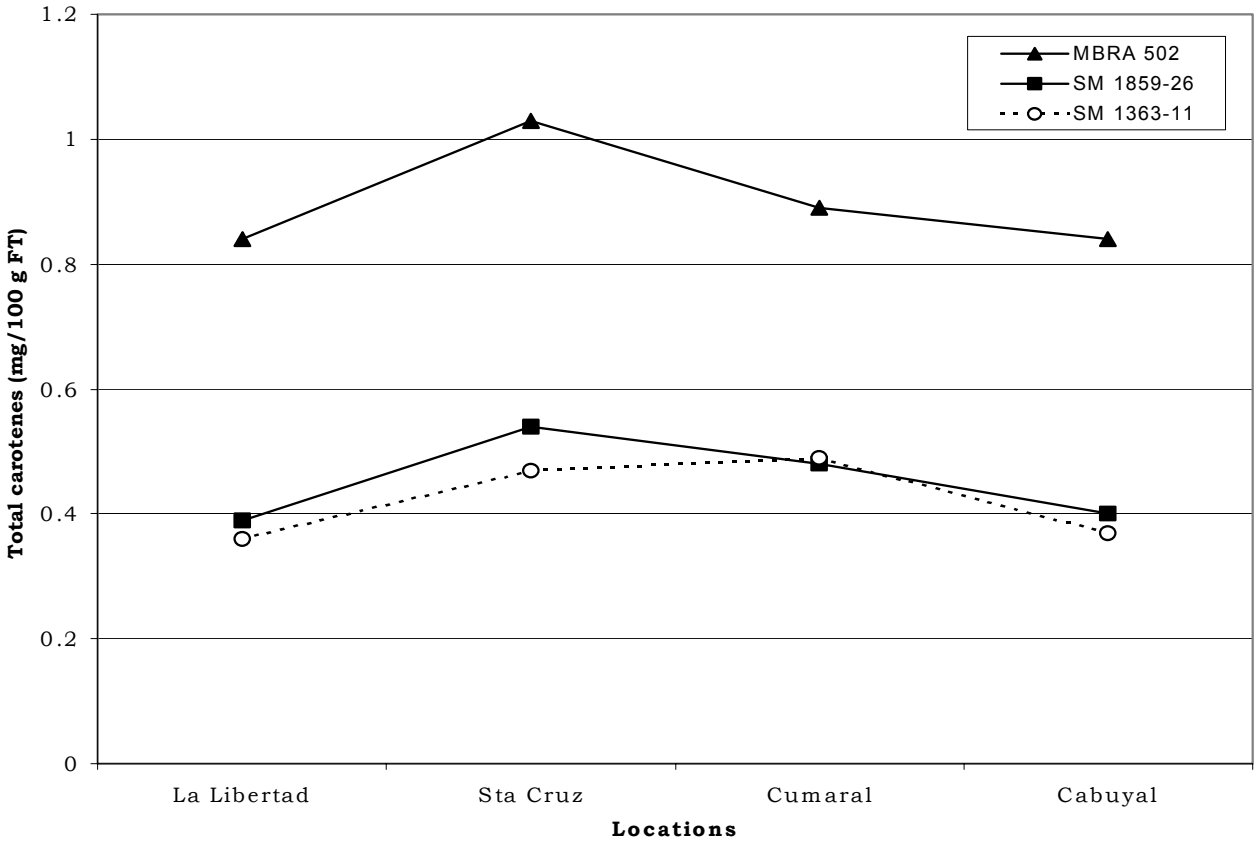

Figure 1.4. Stability of carotene content in the roots from three contrasting clones grown in four different environments in the Acid Soil Savannas of Colombia.

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