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# Introduction

Vitamin A deficiency is still a prevalent problem in many regions of Africa, Asia and Latin America. Vitamin A deficiency is preventable and originates in the unbalanced diets of those populations where malnourishment remains a problem. Vitamin A deficiency has been successfully overcome by supplementation and fortification of different foods. However, these approaches are expensive and solve the problem only temporarily. CIAT has conducted research to evaluate the potential of cassava (*Manihot esculenta* Crantz) as a vehicle for delivering pro-vitamin A carotenoids to those populations chronically deficient in this vitamin. The overall objective of this project 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. This research was supported by the *Biofortification Challenge Program* and financed with resources from DANIDA and USAID.

## **Objectives**

- To determine the range of variation in carotenoid content in accessions from the cassava germplasm collection and elite breeding clones from CIAT.
- To determine the relative importance of different carotenoids present in yellow cassava roots.
- To measure the association between root color and carotenoid contents in cassava.
- To explore alternatives for improving further the concentration of carotenoids already found in cassava.

### Materials and methods

<u>Carotene Extraction</u>. Extraction of the carotenoids present in the root parenchyma followed the procedures described by Safo Katanga, et al. (1). The adjusted protocol included several extractions with acetone and/or petroleum ether 35-65 °C to guarantee total extraction of carotenoid compounds. A sample of 5 g was taken from a root, taken at random from 10 to 11 months old plants (unprocessed check) and after processing the roots. The quantification of pro-vitamin A carotenoids was done by absorption spectrophotometry (for total carotenes) or HPLC (for  $\beta$ -carotene) methodologies.

<u>Quantification of total carotene content</u>. Total carotene content was determined by the absorption spectrophotometry method, using a Shimadzu UV-VIS 160A recording spectrophotometer. Detection was done at  $\lambda$  = 450 nm for root extracts. HPLC system using a YMC-C30 column (250 mm, ID:4.6mm, Waters was used to quantify  $\alpha$ -,  $\beta$ -carotene and lutein. Identification and quantification was done by comparing retention times and uvvis spectra with commercial standards.

**Post-harvest physiological deterioration.** Five commercially sized roots were randomly chosen and analyzed using the method of Wheatley et.al. (2), 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.

<u>*Root color score.*</u> A standard chart depicting nine colors was developed to facilitate root color scoring uniformily.

**Germplasm.** Cassava clones from the germplasm collection at CIAT as well as breeding segregating materials were evaluated. Among the materials evaluated there was a group of 16 clones obtained from the self-pollination of an elite variety from Thailand (Rayong 60 or MTAI 8).

- (1) Safo-Katanga, O., Aboagye, P., Amartey, S.a., and Olaham, J.H. 1984. Studies on the content of yellow-pigmed cassava. In: Terry, E.R. et al. (eds). Tropical Roots crops production and Uses in Africa. IDRC, Ottawa, Canada. pp.103-104.
- (2) Wheatley, C., Lozano, C. Gomez G., 1985. Post-harvest deterioration of cassava roots. In: Cock, J. H., Reyes, J. A. (Eds). Cassava: Research, Production and Utilization. UNDP-CIAT, Cali pp 655-671.

## **Results**

Table 1 describes the range of genetic variability observed among a large number of cassava clones for carotenoid contents in the roots and leaves. The latter exposed considerably higher levels of carotenoids than the roots. Maximum amount of carotenes in roots was 1.069 mg / 100 g of fresh tissue, which demonstrates that yellow cassava roots can be a useful alternative for delivering pro-vitamin A carotenoids to human populations. Correlation between total carotenoid content and root color score was high (0.860 based on a sample of n=788), indicating that a simple inspection of color intensity should suffice for properly identifying carotenoid dense cassava roots.

Table 1. Carotenoids content (mg/100 g of fresh tissue) in cassava roots and

leaves from a large number of cassava clones		
	Roots	Leaves
Sample size	1789	1719
Average	0,246	47.708
Standard deviation	0.135	10.651
Maximum	1.069	96.424
Minimum	0.102	12.049
Skewness	2.590	0.196

HPLC measurements allowed to conclude that a large proportion of the carotenoids present in cassava roots (> 90%) was  $\beta$ -carotene. This is very relevant because of the higher conversion rate of this carotenoid into vitamin A. Negative correlations between carotene content in the roots and post-harvest physiological deterioration was found in different analyses (ranging from -0.123 to -0. 172). This is an interesting finding that would encourage farmers to plant cassava clones with yellow roots due to their reduced or delayed spoiling after being harvested. Although the correlation coefficients are not very high they are promising. Other factors, particularly root dry matter, contribute explaining the variation on physiological deterioration. Figure 1 illustrates the segregation for carotene content in the roots of 16 clones derived from self-pollinating the elite clone Rayong 60. Results suggest that high levels of carotenoids can be obtained even from clones with relatively low levels of carotenes, therefore justifying breeding efforts to increase the levels already attained.



Figure 1. Carotenoids content (mg/100 g of dry tissue) in cassava roots from a cassava clones obtained from self-pollinating the elite cultivar Rayong 60 from Thailand.

#### **Conclusions**

- Enough genetic variability exists for carotenoids content in cassava roots and there are good opportunities to increase it by traditional breeding.
- Yellow cassava roots tend to have reduced/delayed post harvest deterioration.
- Root color is a good indicator of carotene content in cassava roots.