Cloning Carotene Pathway Genes from Cassava

A.F. Salcedo[1] , L.I. Mancilla[2] , D.F. Cortes[3] , J. Beeching[3], P. Chavarriaga[1], and J. Tohme[1]

(1) International Center for Tropical Agriculture CIAT AA 6713, Cali Colombia afsalcedo@cgiar.org
(2) Currently at Universidad Santiago de Cali, Cali Colombia
(3) Currently at Virginia Tech Institute US
(4) University of Bath, UK.

PS6-04

CBN-VI

CIAT
International Center for Tropical Agriculture

Introduction

Carotenoids are present in all photosynthetic organisms, acting in plants as accessory pigments in light harvesting complexes in the thylakoids of chloroplasts. They confer protection against photooxidative stress and are also precursors for abscisic acid. In recent years, genes that code for enzimes involved in carotenoid biosynthesis in several plant species have been cloned and characterized. The regulation of plant carotenoid biosynthesis, as well as efforts to genetically modify staple crops, to increase their b-carotene (provitamin A) content have received considerable attention.

The tissue-specific carotene accumulation could be a result of upstream promoter regulation (Giuliano et al, 1993) or gene structure that code for tissue-specific enzymes (Thorup et al, 2000).

Our work seeks to increase the knowledge on genes coding for specific steps in cassava's carotene metabolism, to understand their regulation in roots. To achieve this goal, combinations of consensus primers were generated to PCR-amplify orthologous sequences of *Phytoene synthase* (*psy*) and *Phytoene desaturase* (*pdes*), which are involved in the first steps of carotene biosynthetic pathway (figure1).

Objective

✓Isolate and characterize *Phytoene synthase* and *Phytoene desaturase* genes from two varieties of cassava with high and low β-carotene content in roots.

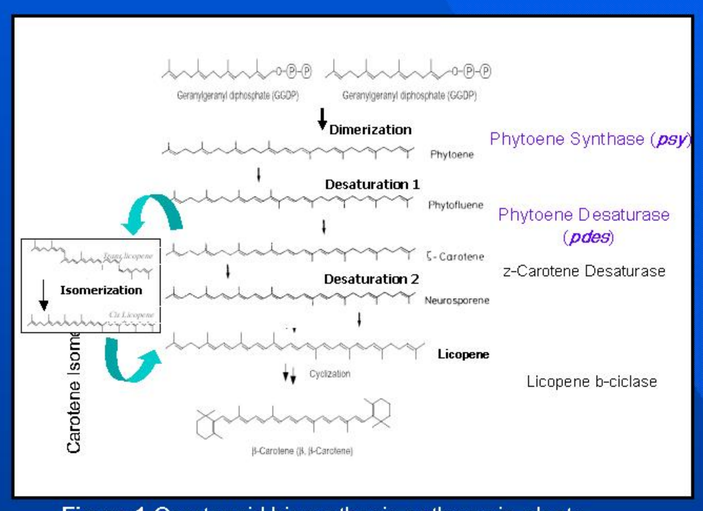

Figure 1 Carotenoid biosynthesis pathway in plants.

Methods

The individuals were cassava cultivars,CM 523-7, whit low root carotene content, and Per 297, whit high root carotene content, which were characterized for its carotene pigment content by HPLC and spectrophotometer analysis. (figure 2)

We extracted genomic DNA from leaves, and used cDNA from fresh roots for PCR amplification.

Combinations of consensus primers were generated by aligning *Phytoene synthase* and *Phytoene desaturase* genes of several plant species to PCR-amplify cassava orthologous sequences.

PCR products were sequenced and analyzed using BlastX algoritm (www.ncbi.nlm.nih.gov). The aminoacid deduced sequence was used to identify conserves domains and motifs in secondary databases.

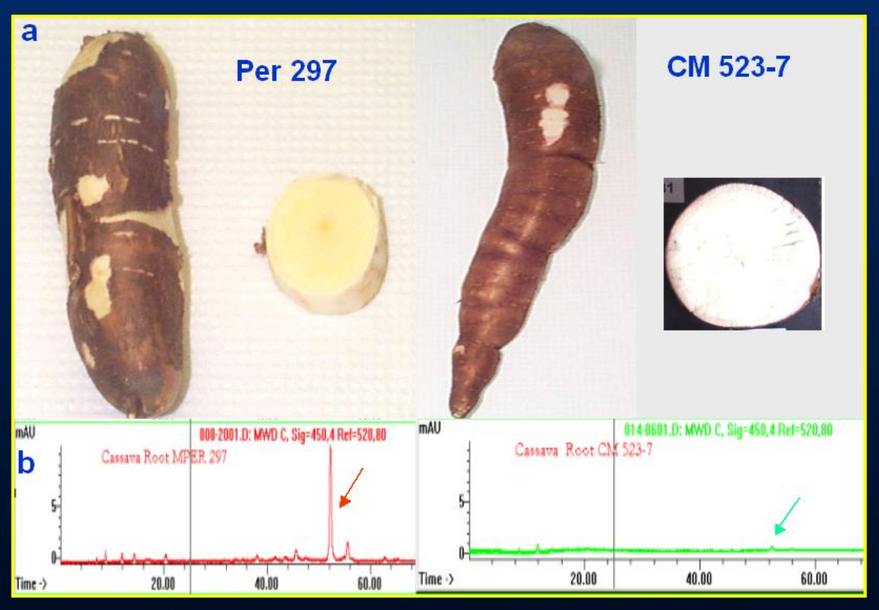

Figure 2 a) Tuber roots from cassava varieties CM 523-7 and MPer 297
b) Comparative b- carotene HPLC analysis. The narrow show retention time for b- carotene.

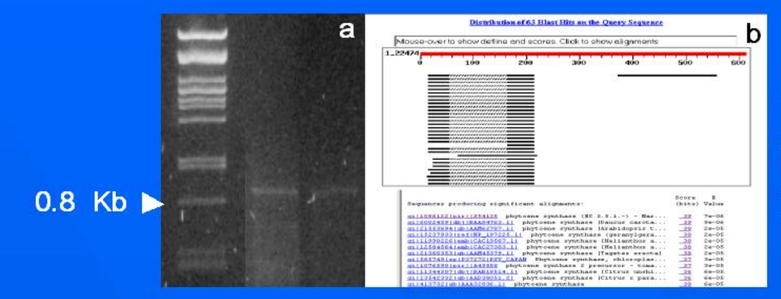

Figure 3
a) PCR amplification of *psy* from genomic DNA
b) Results of BlastX analysis of the sequence.

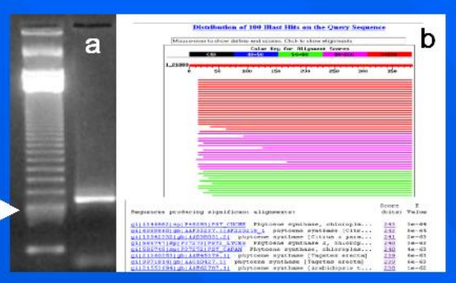

Figure 4 a) PCR amplification of *psy* from cDNA.
b) Results of BlastX analysis of the sequence.

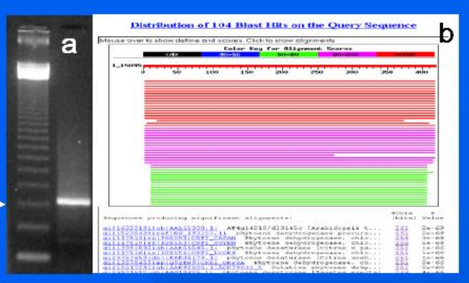

Figure 5 a) PCR amplification of *pdes* from cDNA.
b) Results of BlastX analysis of the sequence.

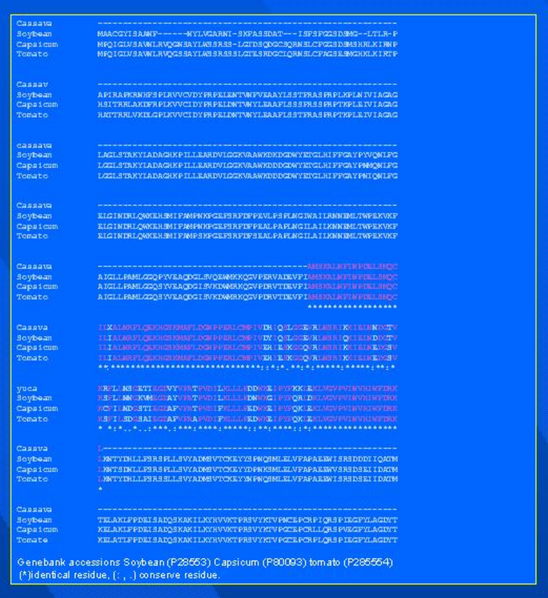

Figure 6 Deduced Cassava´s amino acid sequence alignment of cDNA amplicon for Phytoene desaturase.

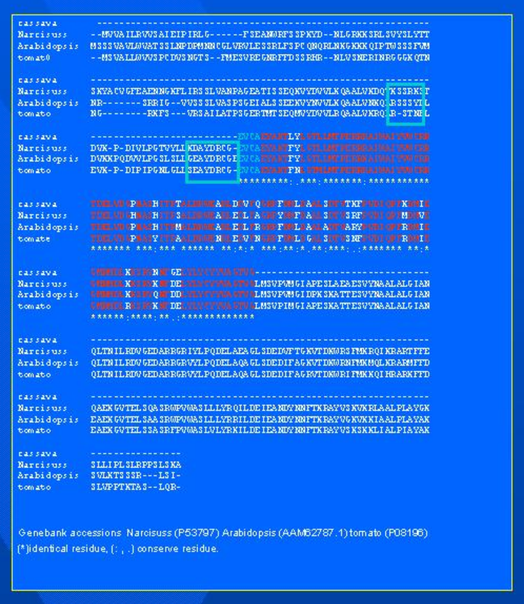

Figure 7 Deduced Cassava´s amino acid sequence alignment of cDNA amplicon for Phytoene synthase. Note, in green, the motif for a signal peptide for chloroplast import.

Results and Discussion

The consensus primers allowed the amplification of orthologus fragments, both in genomic DNA and cDNA. Genomic fragments for *psy* (figure 3a) and cDNA fragments for *psy* and *pdes* (figure 4a and 5a) were obtained testing consensus primers both in Per 297 and CM523-7 cultivars.

Those fragments show high degree of similarity compared to known clones for *psy* and *pdes* (figure 4b and 5b). When we used BlastX algorithm, the genomic sequence of *psy* revealed the presence of intronic sequences (Figure 3b).The deduced amino acid sequence of the orthologus fragment of *pdes* (Figure 6) a showed high degree of homology with exons 7, 8 , 9 and 10 of tomato (Mann 1994).

The deduced aminoacid sequence of the orthologus fragment *psy,* showed a high degree of homology with exons 1, 2 , 3 and 4 of *Zea mays* (Buckner et al, 1996). We also found the conserved motif EVCA, which corresponded to the signal peptide necessary for psy import into the chloroplast (figure 7).

Perspectives

- We constructed a full leght cDNA, cassava root library from cultivar Per 297, with the aim of screening cDNA clones, using psy and pdes PCR products as probes, or by using their cDNA sequences to produce primers for RACE (Rapid Amplification of cDNA Ends) technology, to isolate *psy* and *pdes* full leght cDNAs.
- Screening of genomic cassava libraries from CM 523-7 and Per 297 with *psy* and *pdes* orthologous fragments will allow cloning and comparison of promoter sequences.
- Test new primer sets to amplify fragments of other carotene pathway genes like z-Carotene Desaturase, Licopene b-ciclase, and carotene isomerase

References

BARTLEY G.E., et al, 1994. Annu.Rev.Plant Physiol. Plant. Mol. Biol. 45: 287-301
BUCKNER, B., et al 1996. Genetics 143:479-488.
CUNNINGHAM, F.X.& J..GRANTT.1998. Annu. Rev. Plant Physiol. Plant. Mol. Biol. 49: 557-583.
GIULIANO, et al. 1993. The Plant Cell 5:379-387.
JOS, J. et al, 1990. Journal of Root Crops 16: 5-11.
MANN, et al, 1994. Plant Molecular Biol 24: 429-434.
THORUP, et al. 2000. PNAS USA 97: 11192-11197.