Searching for a molecular explanation of differences in carotenoid content of cassava roots: Identification and expression profile of a Carotenoid Cleavage Dioxygenase I (CCD 1) gene

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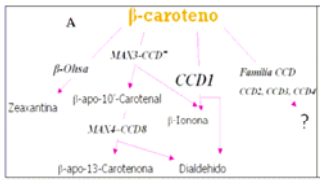
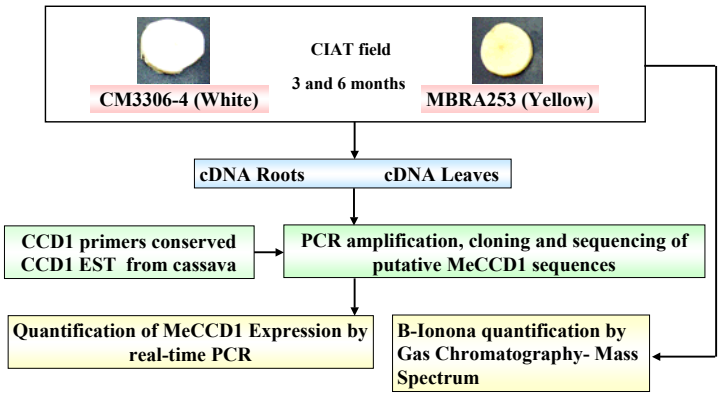
INTRODUCTION

The molecular basis of the differential accumulation of β-carotene in cassava tuberous roots are not yet known. Catabolism of carotenes may partially explain those differences. In some plant species it has been confirmed that the gene Carotenoid Cleavage Dioxigenase 1 (CCD1) catabolizes β-carotene generating β-Ionona :



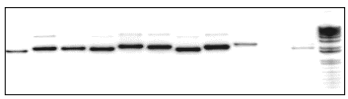
To determine if there was a relationship between the activity of a putative *Manihot esculenta* CCD1 gene (MeCCD1) and the carotene accumulation in cassava roots and leaves of white- and yellow-rooted cultivars, we measured its expression levels in both organs. Simultaneously, we also measured and quantified β-Ionona by means of Gas Chromatography with Mass Spectrometry.

METHODS



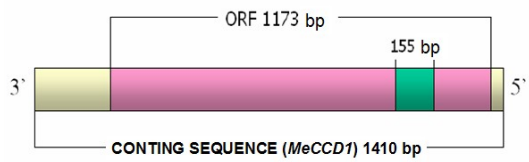
RESULTS AND DISCUSSION

• Isolation of putative MeCCD1 sequences



CCD1 amplification of five cDNA clones isolated from yellow roots.

Five fragments of cDNA generated a contig of 1410 bp (MeCCD1), with high homology to CCD1.



Fragment used for real-time RT-PCR analysis

The MeCCD1 putative protein displayed an ORF of 468 amino acids, and it was highly conserved with the protein family Carotenoid_ Oase.

• Quantification of MeCCD1 mRNA levels

MeCCD1 mRNA levels did not show significant differences between white and yellow roots.

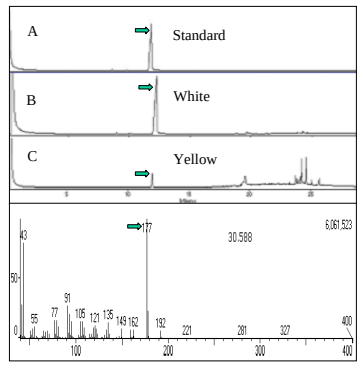
In leaves from the yellow variety, the MeCDD1 expression was higher that in the white variety:

Relative MeCCD1 mRNA levels in leaves

Leaves	3 months	6 months	Average
MBRA253 "yellow"	8.74	8.98	8.86
CM3306-4 "white"	3.93	3.99	3.96

• Detection and confirmation of ß-Ionona in roots

A methodology was developed to detect and confirm ß-Ionona in cassava roots

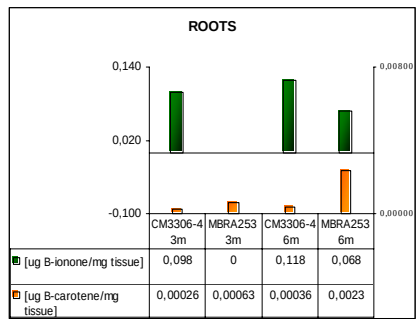


• ß-Ionona detection by Gas Chromatography in yellow and white roots

• Confirmation of ß-ionona detection by Mass Spectrometry

• Quantification of ß-ionona in roots

In white roots, the levels of β-ionona were statistically superior to those of the yellow, while the β-carotene levels (Arango et al 2005) were higher in yellow that in white roots:



Results suggest an inverse relationship between the contents of β-carotene and β-Ionona, which may partially explain differential accumulation of β-carotene in the storage roots of cassava. The expression of MeCCD1 in roots do not explain these findings.

We suggest that β-Ionona is produced in cassava possibly due to β-carotene catabolism.

PERSPECTIVES

• To evaluate the expression of different members of the CCD family.

• Cloning of CCD candidates and functional validation by gene silencing in white cassava roots.

Date prepared: October 2007