

Transgenic cassava with bacterial genes of the carotenoid biosynthetic pathway accumulate higher levels of phytoene, lutein and α and β -carotene.

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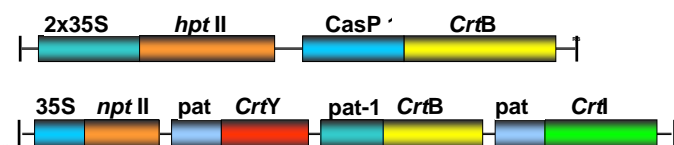


INTRODUCTION

CIAT and the University of Freiburg (Germany) are inserting into cassava a series of combinations of promoters and bacterial genes to increase the content of β -carotene in roots. This effort is part of a HarvestPlus strategy to augment the level of micronutrients in staple foods to improve human nutrition.

MATERIALS AND METHODS

- Constructs in pCAMBIA1305.2-based vectors:
 - pCAS-Phyt with promoter from cassava roots
 - pPat-Erwinia II with promoters from potato (Patatin) provide a mini-pathway for carotene synthesis

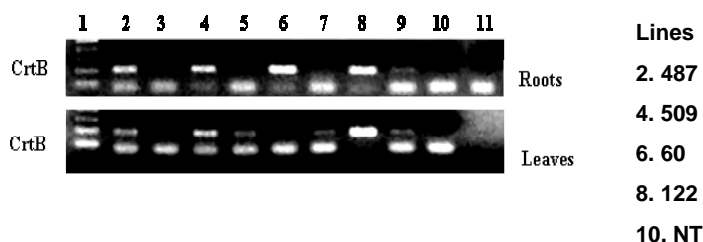


- Cassava clones:
 - 60444, MCol2215, CM3306-4
- Tissues:
 - Friable embryogenic callus and cotyledons from somatic embryos
- Agrobacterium strains:
 - AgI1, LBA4404

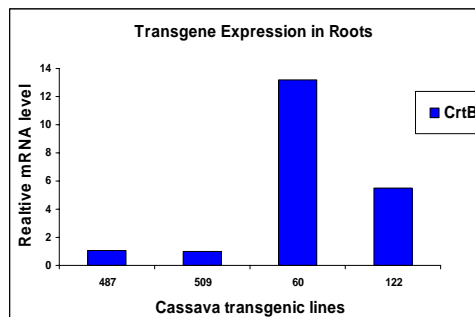
RESULTS AND DISCUSSION



• Yellow calli and roots of transgenic cassava lines transformed with pCas-Phyt. Fifteen lines are growing in the green house.



• Real-time PCR amplification of expressed *crtB* in roots and leaves of transgenic lines transformed with pCas-Phyt.



• Real-time PCR quantification of expression of the *crtB* gene in roots of cassava lines transformed with pCas-Phyt.

Line	Phytoene ($\mu\text{g/g FW}$)	Total carotenes ($\mu\text{g/g FW}$)
487	6,21	4,55
509	nd	nd
60	0-2,89	0,29-1,27
122	17,4	4,29
NT	Undetectable	1,27

• HPLC estimation of carotenoids in roots from in vitro transgenic cassava plants transformed with pCAS-Phyt



Line	α -carotene ($\mu\text{g/g FW}$)	β -carotenes ($\mu\text{g/g FW}$)	Lutein ($\mu\text{g/g FW}$)
115	2,44	1,92	2,19
85	7,04	6,14	3,05
NT	Undetectable	Undetectable	Undetectable

• Regenerating in vitro plants from two lines, 85 and 115, out of eight transgenic lines established in vitro. HPLC estimation of carotenoids in these two transgenic lines transformed with pPat-Erwinia II.

PERSPECTIVES

Analyzing carotene content in tuberous roots from greenhouse- and field-grown plants. Over 100 new putative transgenic lines are currently growing in vitro.

Regenerating and testing more plants with promoters from Cassava roots (Extensin), Sugar Beets and Yams, driving the expression of the same *crtB* gene, or the mini-pathway, to compare strength and tissue specificity of promoters.