

## METABOLIC ENGINEERING OF POTATO TUBER CAROTENOIDS THROUGH TUBER-SPECIFIC SILENCING OF LYCOPENE EPSILON CYCLASE

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Potato is a major staple food, and modification of its provitamin content is a possible means for alleviating nutritional deficiencies. beta-carotene is the main dietary precursor of vitamin A. Potato tubers contain low levels of carotenoids, composed mainly of the xanthophylls lutein, antheraxanthin, violaxanthin, and of xanthophyll esters. None of these carotenoids have provitamin A activity.

In order to redirect carotenoid biosynthesis in potato tubers, we silenced the first dedicated step in the beta-epsilon-branch of carotenoid biosynthesis, lycopene epsilon cyclase (*LCY-e*). This was achieved by cloning an antisense *LCY-e* fragment under the control of the patatin promoter. This promoter was able to drive tuber-specific expression of the *GUS* reporter gene and of the *LCY-e* silencing transcript. Real time measurement of endogenous carotenoid gene expression confirmed the silencing of *Lcy-e*. Silenced tubers showed significant increases in beta-beta-carotenoid levels, with beta-carotene showing the maximum increase (up to 14-fold). This was not accompanied by a decrease of the main b-e-carotenoid, lutein, suggesting that *LCY-e* is not rate-limiting for lutein accumulation. Total carotenoid levels increased up to 2.5-fold. Increases in expression were observed for several genes of the pathway (*CrtISO*, *LCY-b* and *ZEP* and, for one line, also *PSY1* and *CHY1*). All of the above effects were observed in tubers, but not in leaves, confirming that gene silencing, and its phenotypic consequences, remained confined to tubers. The data suggest that epsilon-cyclization of lycopene is a key regulatory step in potato tuber carotenogenesis. Upon tuber-specific silencing of the corresponding gene, expression of several other genes in the pathway is modified, and b-b--carotenoid and total carotenoid levels are increased.