Regulatory mechanisms of carotenoid biosynthesis in potato tubers

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Introduction

Isoprenoid biosynthetic pathways provide a wide-range of metabolites essential for plant development and storage organ food quality. Amongst them are carotenoids, photosynthetic pigments and important micronutrients with provitamin A activity. Globally, it was estimated that 140–250 million children under five years of age are affected by vitamin A deficiency, so understanding the regulation of isoprenoid metabolic networks is fundamental in producing foods with enhanced nutrient content.

Many isoprenoids are synthesized in the plastid, however in potato tubers there are gaps in our knowledge about how this biosynthesis is regulated. Previous transgenic work, whilst providing proof of principle that gene expression changes can bring about improvements (Shewmaker *et al.* 1999; Ducreux *et al.* 2005), have not helped us understand the different tiers of regulation that can be addressed to make further improvements in isoprenoid levels.

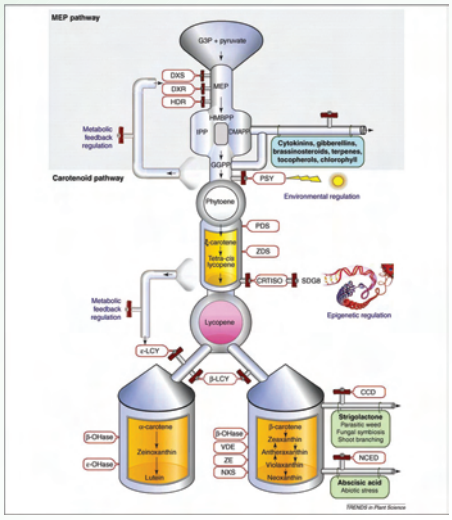

Figure 1. Major reactions in the higher plant carotenoid biosynthetic pathway showing enzymes, carotenoids and their precursors (pipes), carotenoid sinks (barrels), carotenoid- derived signalling hormones (green signs) and other MEP isoprenoid-derived metabolites (blue sign). The windows displayed within the chrome pipes indicate abundant carotenoid pigments found in photosynthetic tissues and also represent key nodes for regulation in the pathway. Carotenoid biosynthesis is modulated by environmental factors (light), chromatin modification and metabolic feedback regulation. The side funnels represent examples of metabolic feedback control mechanisms acting upon biosynthetic gene expression as a result of altered PSY and CRTISO enzymatic activity, respectively. First, the bottleneck in phytoene biosynthesis is regulated by PSY and its overexpression increased DXS and DXR mRNA levels post-transcriptionally in etiolated tissues. Second, loss-of-function CRTISO mutants show reduced eLCY transcript levels in etiolated tissues. Abbreviations: bLCY, b-cyclase; bOHase, b-hydroxylase; CCD, carotenoid cleavage dioxygenase; CRTISO, carotenoid isomerase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxyxylulose-5-phosphate synthase; eLCY, e-cyclase; eOHase, e-hydroxylase; GGPP, geranylgeranyl diphosphate; HDR, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase; NCED, 9-cis-epoxycarotenoid dioxygenase; NXS, neoxanthin synthase; PDS, phytoene desaturase; PSY, phytoene synthase; SDG8, histone methyltransferase; VDE, violaxanthin de-epoxidase; ZDS, z-carotene desaturase; and ZE, zeaxanthin epoxidase (Reproduced from Cazzonelli C.I. and Pogson).

Aims of the Project

1. Identify the plastid type involved in isoprenoid metabolism in potato tubers

HOW- using GFP and RFP tagged carotenoid biosynthesis related proteins localized to the plastids.

WHY- protein import to the plastid is likely to be a bottleneck in isoprenoid biosynthesis in transgenically manipulated plants.

2. Is carotenoid accumulation regulated by degradation?

HOW- A carotenoid cleavage diooxygenase (*CCD4*) will be introduced into naturally high carotenoid species of potatoes.

WHY- *CCD 4* was found to have enhanced expression in white-fleshed potato tubers and lowered expression levels in carotenoid accumulating yellow-fleshed potato tubers (Campbell *et al.* 2010).

Results

RFP and GFP tagged fusions of Gateway binary vectors were created using the backbone of pK7FWG2 and pK7RWG2 (VIB, UGENT) together with the coding sequences of phytoene synthase *PSY 2* and beta carotene hydroxylase *CrtRB2* from potato (see Figure 2).

These constructs were then used for
- transient expression in *Nicotiana benthamiana*, *Solanum tuberosum* cv Desiree and *Solanum phureja* cv Mayan Gold and Inca Sun
- stable transformation in potato tissue culture explants (*Solanum tuberosum* cv Desiree).

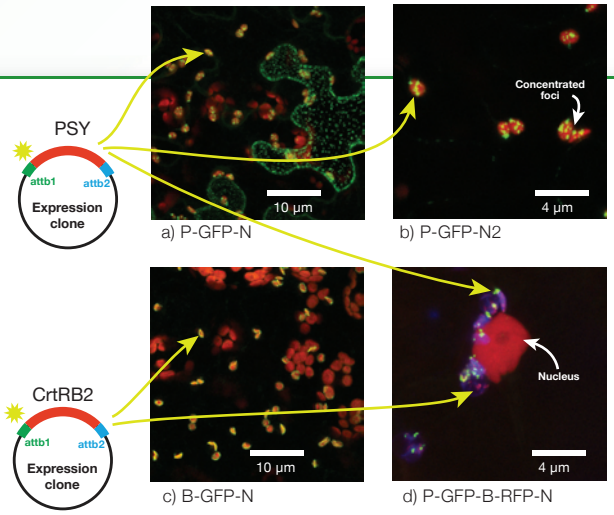

Figure 2 shows expression of the *PSY*-GFP (P-GFP) and *CrtRB2* – RFP (B-RFP) proteins at 3 days post inoculation (dpi) in *Nicotiana benthamiana* leaf tissues. The arrows indicate the presence of the GFP and RFP signal localized to the plastids, in the leaf mesophyll cells, apparently accumulating in concentrated foci in the case of *PSY*.
a)-c) : Green - *PSY*/*CrtRB2*; Red - chlorophyll d) : Green - *PSY*; Red - *CrtRB2*; Blue - chlorophyll

Discussion

All constructs show plastid localization, as expected. However, because PSY localizes as concentrated foci with a stronger outer membrane fluorescence signal, it is worth investigating if it accumulates in a suborganellar complex or compartment.

Conclusion

These preliminary results indicate that the destination vectors can be used for further work in identifying the types of plastids and regulation related mechanisms of carotenoid synthesis in potato tubers, both by stable and transient transformation.

Further Work

Constructs with the OR *orange* cauliflower gene that facilitates the accumulation of high levels of beta-carotene in tissues normally lacking carotenoids (Lu *et al.* 2006) and CCD4 (*carotenoid cleavage diooxygenase 4*) coding sequences will be generated and assayed for an influence in the levels of carotenoids in potato tubers.

Analysis of the stable trangenics generated with the presented constructs (confocal microscopy, HPLC and molecular analysis).

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