Transgenic manipulation of tuber carotenoid content in *Solanum phureja* and *Solanum tuberosum*

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Introduction

Carotenoids are essential components of all photosynthetic organisms and are secondary metabolites in some plant organs (flowers, some tubers). Certain carotenoids play an important role in human nutrition (e.g. β-carotene has pro-vitamin activity, lutein and zeaxanthin protect against macular degeneration). Vitamin A deficiency is a widespread problem in developing countries, but also in poor populations, elderly, heavy drinkers and smokers of industrialised nations. The World Health Organisation estimates that improving vitamin A nutrition could prevent more than 2 million deaths every year.

The aim of this project is to understand the factors that lead to carotenoid accumulation in potato tubers. We work to be able to develop potato tubers with enhanced carotenoid content and balance, improving the quality of a staple part of our diet.

Solanum phureja is a diploid cultivated potato. As well as having significance in potato breeding as a source of valuable resistance genes to several biotic and abiotic stresses, accessions of this species produce tubers that have enhanced culinary and nutritional properties. However, no published transformation protocol is available. We used a range of *Solanum phureja* accessions available at SCRI and empirically explore a wide range of protocols and variables in order to develop a transformation protocol.

Approach: We shall use a transgenic approach to engineer the carotenoid metabolic pathway - using either an antisense approach or by increasing the flux through the pathway by over-expressing bacterial genes in the potato tuber.

Around 40 transgenic lines for each construct were obtained in Desiree. Here we present a detailed analysis of the *Crt B* (bacterial phytoene synthase) transgenic lines in both *Solanum phureja* DB337/37 and *Solanum tuberosum* cv. Desiree.

Analysis of *Solanum tuberosum* cv. Desiree Crt B lines

Solanum tuberosum cv. Desiree is easily transformed

Comparison of the effects of transformation in the two systems

Tubers from some *Solanum phureja* accessions are rich in lutein and zeaxanthin

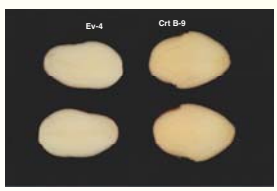
Understanding the regulation of the carotenoid pathway

Strong expression of the transgene (*crt B*, phytoene synthase) in the tubers of some transgenic lines.

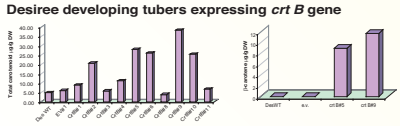
Carotenoid content increased up to 7 fold compare to wild type and empty vector lines.

Accumulation of β-carotene

Desiree mature tubers



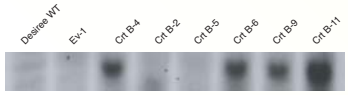
Desiree developing tubers expressing *crt B* gene



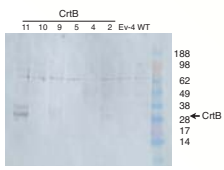
Carotenoid acetone extracts

Crt B: developing potato tubers expressing *crt B* (phytoene synthase) gene. Controls: WT, Wild type. Ev, empty

Northern Blot

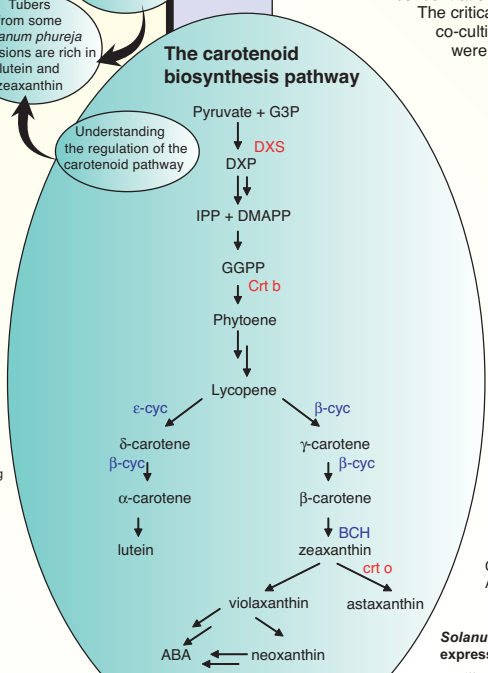


Western Blot Crt B antibody



Samples	Carotenoids g/g DW [Total]	Neo	Vio	Ant	Lut	Zea	B-car
Des WT	4.98	0.75	1.49	0.45	0.9	0.15	0
EV-3	5.89	0.88	1.41	0.59	1.06	0.18	0
crt B-2	20.67	0.83	6.61	0.41	4.75	0.21	5.37
crt B-4	11.26	0.79	4.39	0.34	2.03	0.23	0.11
crt B-5	28.04	1.12	7.57	0.84	6.45	0	8.97
crt B-9	38.32	1.15	9.2	0.77	11.88	0.77	11.11
crt B-10	25.53	1.28	6.38	1.02	7.15	0	5.36
crt B-11	6.76	0.74	1.69	0.2	1.28	0.14	2.03

The carotenoid biosynthesis pathway



Constructs

pBI vector with kanamycin resistance

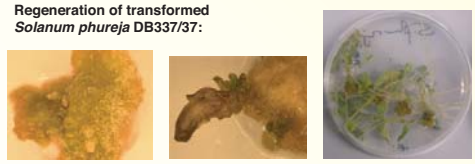
Patatin promoter	Transit peptide	Sense contructs Crt b DXS	Poly A

Patatin promoter	Antisense contructs β-carotene hydroxylase	Poly A

Solanum phureja DB337/37 transformation

A transformation protocol was developed to transform *Solanum phureja* DB337/37: experiments to optimise regeneration were carried out by varying antibiotic and hormone concentration, temperature, Agrobacterium concentration, duration on each media. The critical point was found to be the amount of Agrobacterium used and the co-cultivation period. Although inefficient, 23 crt B lines were obtained, 6 lines were selected for detailed analysis.

Regeneration of transformed *Solanum phureja* DB337/37:



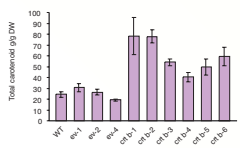
Callus stage | Shooting stage | Rooting stage

Analysis of developing tubers expressing *crt B* (phytoene synthase) gene

Sample	Total carotenoids g/g DW	SD
WT	24.4	2.65
ev-1	30.6	3.85
ev-2	26.6	2.42
ev-3		
ev-4	19.3	0.8
crt b-1	78.1	17.07
crt b-2	77.8	5.98
crt b-3	54.1	3.27
crt b-4	40.5	4.26
crt b-5	49.7	7.36
crt b-6	59.5	8.67

Carotenoid content increase up to 3 fold compare to wild type and empty vector lines. Accumulation of β-carotene, lutein, violaxanthin and antheraxanthin.

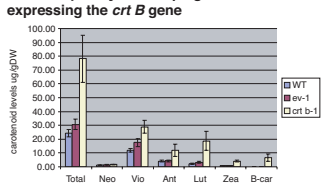
***Solanum phureja* developing tubers expressing the *crt B* gene**



Carotenoid levels g/g DW

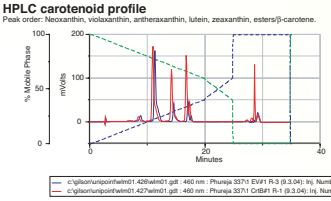
Samples	Total	Neo	Vio	Ant	Lut	Zea	β-car
333WT rep1	25.93	1.04	12.19	4.41	2.59	0.78	0.00
333WT rep2	25.99	1.04	13.00	3.64	2.60	0.78	0.00
333WT rep3	21.38	1.28	10.69	4.49	1.92	0.64	0.00
ev-1 rep1	26.60	1.33	14.37	5.05	2.66	0.80	0.00
ev-1 rep2	31.06	1.55	18.02	3.73	3.42	0.62	0.00
ev-1 rep3	34.27	1.71	20.22	4.11	3.77	1.03	0.00
crt b-1 rep1	80.26	1.61	28.09	16.85	16.85	4.82	6.42
crt b-1 rep2	94.04	1.88	33.86	8.46	26.33	3.76	9.40
crt b-1 rep3	60.11	1.20	24.64	10.82	13.22	3.61	4.21

***Solanum phureja* developing tubers expressing the *crt B* gene**



HPLC carotenoid profile

Peak order: Neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, esters/β-carotene.



Conclusion / Future Work:

- Development of a transformation protocol for diploid *Solanum phureja* DB337/37 (high carotenoid content)
- Engineering of high β-carotene tubers by overexpressing Erwinia *crt B* gene.
- Analysis of the second generation of tubers.
- Transformation of high carotenoid content transgenic Desiree crt B 9 with ZEP (zeaxanthin epoxidase).
- Insight into how the pathway is regulated and linked with carotenogenesis in potato.
- Use of microarray in conjunction with transgenic to hunt for regulatory genes.

Acknowledgements
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