Characterisation and transgenic modification of carotenogenesis during tuber development and storage in potato

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Introduction

As the World's 4th most important source of calories, the nutritional quality of potato tubers is an area of key interest to plant scientists. The health benefits of different carotenoids become increasingly evident and so the types and amounts of carotenoids in potato tubers assume more significance.

Carotenoids in potato tubers

Several studies have characterised tuber carotenoid content from a range of potato germplasm and shown that *Solanum tuberosum* and *Solanum phureja* exhibit a wide (over 20-fold) variation in tuber carotenoid content (Brown *et al.*, 1993, Lu *et al.*, 2001). The total tuber carotenoid content in some *Solanum stenotomum* X *Solanum phureja* crosses reached up to 1435 µg/100g FW compared with typical carotenoid levels of ca. 10,000 µg/100g FW in carrot taproot (Simon and Wolff, 1987). Tuber carotenoid content in *Solanum tuberosum* cultivars has also been measured (Breithaupt and Bamedi, 2002; Iwanzik, 1983). For example yellow-fleshed cultivars contain 58-175 µg/100g FW carotenoid and white fleshed cultivars contain 38-62 µg/100g FW carotenoid. The main carotenoids of *Solanum tuberosum* tubers are violaxanthin, antheraxanthin, lutein and zeaxanthin although the ratios of these carotenoids vary between cultivars. Carotenoid esters in tubers from some *S. tuberosum* cultivars can reach significant levels (up to 131 µg/100g FW Breithaupt and Bamedi, 2002).

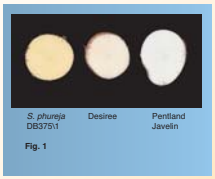

Fig. 1

Aims of the project

By comparing different potato germplasm, we wish to gain insights into the factors that control tuber carotenoid content.

We wish to exploit this knowledge to be in a position to rationally tailor tuber carotenoid content.

We wish to develop protocols for the transformation of high tuber carotenoid accessions of *S. phureja*.

Results

The levels of carotenoids during tuber development and storage were compared in a high carotenoid accumulating *S. phureja* accession (DB375\1) with two *S. tuberosum* cultivars (Pentland javelin and Desiree) that accumulate lower levels of tuber carotenoid. In *S. phureja* tubers at maturity the major carotenoids were zeaxanthin, antheraxanthin and violaxanthin. Following 9 months storage at 4°C the levels of zeaxanthin and antheraxanthin decreased whereas the level of lutein increased however, overall there was only a small decrease in total carotenoid content.

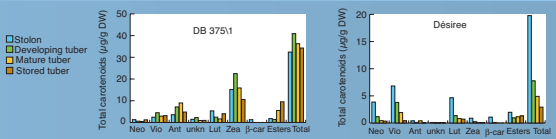

Fig. 2 Carotenoid content during tuber development and storage

Expression profiling

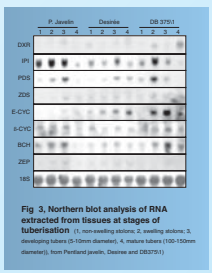

Fig 3, Northern blot analysis of RNA extracted from tissues at stages of tuberisation (1, non-swelling stolons; 2, swelling stolons; 3, developing tubers (5-10mm diameter), 4, mature tubers (100-150mm diameter)), from Pentland javelin, Desiree and DB375\1)

In order to explore reasons for the wide variation in tuber carotenoid content, the expression patterns of the major genes encoding the enzymes of the carotenoid biosynthetic pathway were compared. Significant differences in the profiles were detected, suggesting that transcriptional control or mRNA stability gives rise to the large differences in tuber carotenoid content. In particular, there was an inverse trend between the level of zeaxanthin epoxidase transcript level and tuber carotenoid content in a range of potato germplasm.

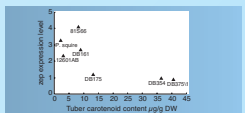

Fig 5 Quantitative RT-PCR analysis using primers specific for the potato tuber zeaxanthin epoxidase sequence of RNA extracted from mature tubers of a range of potato germplasm (accession code indicated on figure) plotted against the total tuber carotenoid content. Expression levels are expressed relative to the level determined in tubers of DB375\1.

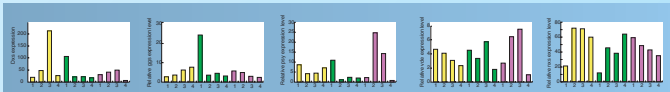

Fig. 4 Quantitative RT-PCR analysis of RNA extracted from tissues at stages of tuberisation (as in Fig.3)

The inverse relationship between the level of zeaxanthin epoxidase and total tuber carotenoid content reflects the effects of down-regulation of zeaxanthin epoxidase reported by Romer et al., 2002 - a major stimulation of the entire carotenogenic pathway. Does this reflect an attempt by the plant to maintain homeostasis in the level of carotenoids and apocarotenoids downstream of zeaxanthin ?

Does ZEP map to the Y locus ?

The Y locus, exerting a major influence on tuber colour has been mapped to chromosome 3 (Thorup et al., 2000). Using a cleaved amplified polymorphism (CAPS) assay, we mapped ZEP to chromosome 2. However other copies of the ZEP gene may be located elsewhere in the potato genome.

Microarray analysis of the effects of *crtb* over-expression in potato tubers

As there is a large increase in tuber carotenoid content in the *crtb* tubers, we have the opportunity to investigate the mechanisms that are associated with carotenoid storage.
Expression profiling was carried out using developing tubers from two *crtb* over-expressing lines using a total of 5 independent replicates, compared with 5 independent tubers from empty-vector controls. Each spot represents a gene that shows a significant change in expression between the transgenic and control (upper green line = 2-fold up-regulated, lower green line = 2- fold down-regulated).

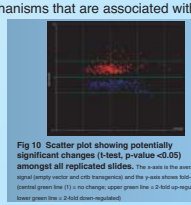

Fig 10 Scatter plot showing potentially significant changes (t-test, p-value <0.05) amongst all replicated slides. The x-axis is the average signal (empty vector and crtb transgenics) and the y-axis shows fold-change (central green line (1) = no change; upper green line = 2-fold up-regulated; lower green line = 2-fold down-regulated)

Transgenic modification of tuber carotenoid content.

A wide range of constructs have been assembled either to down-regulate potato genes encoding the biosynthetic enzymes or to over-express bacterial or algal genes. We are currently analysing the effects of these transgenes.
Results are shown for the effects of over-expressing the *Erwinia crtb* gene, encoding phytoene synthase in *Solanum tuberosum* cv Desiree. The construct was driven by the patatin promoter and the RubisCo small subunit plastid targeting sequence was used.

Total carotenoid levels increased ca. 7-fold compared with empty vector transformed controls. The major carotenoid that accumulates in these tubers is β-carotene, normally present at neglible levels in potato tubers.

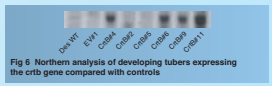

Fig 6 Northern analysis of developing tubers expressing the crtb gene compared with controls

Fig 7 Total carotenoid levels in developing potato tubers expressing the crtb gene compared with controls

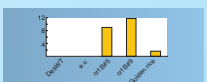

Fig 8 β-carotene levels in developing potato tubers expressing the crtb gene compared with controls

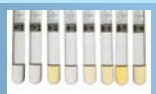

Fig 9 Acetone extracts of freeze dried developing potato tubers expressing the crtb gene compared with controls

Transformation of high tuber carotenoid *Solanum phureja*

In order to increase carotenoid content beyond that previously seen for potato germplasm we are transforming high tuber carotenoid accessions of *S. phureja* with the *crtb* construct.
Protocols for the transformation of *S. tuberosum* were ineffective when applied to *S. phureja* and we have developed a new transformation protocol.
Although inefficient, *S. phureja* transgenic lines have been generated and are currently being assessed.

Fig 11 Regeneration of transformed *Solanum phureja*.

Work in progress

Detailed analysis of a wide range of potato transgenics with modified carotenoid content.

Development of *S. phureja* transgenics with modified carotenoid content.

Stacking of transgenes to obtain larger effects on tuber carotenoid content.

Microarray analysis of the effects of expressing transgenes in the tuber.

Analysis of novel genes revealed by microarray analysis.

References

Breithaupt DE, Bamedi A. 2002. Carotenoids and carotenoid esters in potatoes (*Solanum tuberosum* L.): New insights into an ancient vegetable. *Journal of Agricultural and Food Chemistry* 50, 7175-7181.

Brown CR, Edwards CG, Yang CP, Dean BB. 1993. Orange flesh trait in potato: Inheritance and Carotenoid Content. *Journal of the American Society for Horticultural Science* 118, 145-150.

Iwanzik W, Tevini M, Stute R , Hilbert R. 1983. Carotinoidgehalt und zusammensetzung verschiedener deutscher Kartoffelsorten und deren Bedeutung fur die Fleischfarbe der Knolle. *Potato Research* 26, 149-162.

Lu W, Haynes K, Wiley, E and Clevidence B. 2001. Carotenoid content and color in diploid potatoes. *Journal of the American Society of Horticultural Science* 126, 722-725.

Romer S, Lubeck J, Kauder F, Steiger S, Adomat C, Sandmann G. 2002. Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co- suppression of carotenoid epoxidation. *Metabolic Engineering* 4, 263-272.

Simon PW, Wolff XY. 1987. Carotenes in typical and dark orange carrots. *Journal of Agricultural and Food Chemistry* 35, 1017-1022.

Thorup TA, Tanyolac, B, Livingstone KD, Popovsky S, Paran I, Jahn M. 2000. Candidate gene analysis of organ pigmentation loci in the Solanaceae. *Proceedings of the National Academy of Sciences USA* 97, 11192-11197.

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