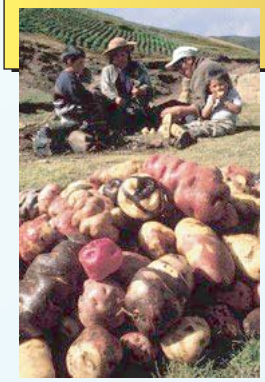
Carotenoid accumulation during potato tuber development and storage

W L Morris, L Ducreux, D W Griffiths, D Stewart, H V Davies and M A Taylor
Quality, Health & Nutrition, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA.

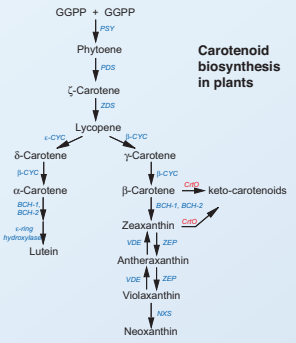
Scottish Crop Research Institute



Introduction

The health benefits associated with the availability of carotenoids in the human diet are becoming increasingly apparent. Carotenoids cannot be synthesised by vertebrates and therefore need to be provided via dietary intake. There would be an undoubted benefit to health therefore if the carotenoid content and balance could be improved in a staple food such as potato.

Tuber flesh colour can range from white through to orange and is a direct consequence of the presence of carotenoids. The molecular basis for this wide variation in tuber carotenoid content is not understood. The aim of this study is to compare carotenogenesis in white, yellow and orange-fleshed potato germplasm in order to reveal factors affecting carotenoid levels. This knowledge will then be used to identify possible targets for a transgenic approach.

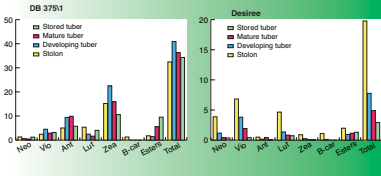


Carotenoid biosynthesis in plants

Results

Comparison of carotenoid profiles during tuber development and storage

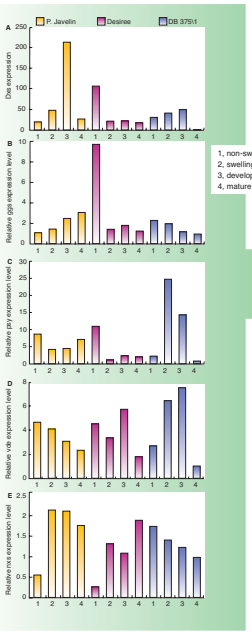
The levels of carotenoids during tuber development and storage were compared in a high carotenoid accumulating *S. phureja* accession (DB375\1) with two *S. tuberosum* cultivars (Pentland Javelin and Desiree) that accumulate lower levels of tuber carotenoid. In *S. phureja* tubers at maturity the major 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.



Carotenoid content during tuber development and storage

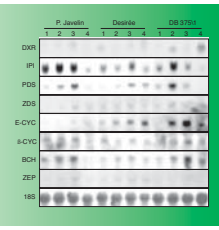
Biosynthetic gene expression profiling

The transcript levels of the genes encoding carotenogenic enzymes have been profiled in a range of germplasm during tuber development. Significant differences in the expression profiles were detected; suggesting that transcriptional control or mRNA stability gives rise to the large variation in tuber carotenoid content. Surprisingly, an inverse relationship between zeaxanthin epoxidase transcript level and total tuber carotenoid content was detected.



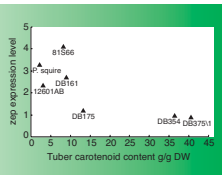
1, non-swelling stolons;
2, swelling stolons;
3, developing tubers (5-10mm diameter),
4, mature tubers (100-150mm diameter),

Northern blot analysis of RNA extracted from tissues at stages of tuberisation



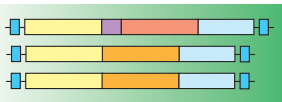
Quantitative RT-PCR analysis of RNA extracted from tissues at stages of tuberisation

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.)



Creation of constructs for plant transformation

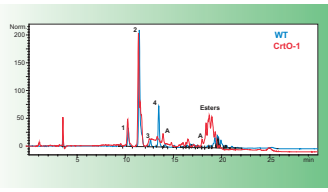
Partial cDNAs of the genes involved in carotenoid biosynthesis were cloned from *S. phureja* DB375\1 tuber cDNA by RT-PCR using primers designed to conserved regions of publicly available sequences. These fragments were used as probes in gene expression studies. Lycopene-ε-cyclase (ε-cyc) and lycopene-β-cyclase (β-cyc) clones were used in the assembly of antisense constructs. An algal (*Haematococcus pluvialis*) *crtO* over-expresser construct was also produced. The constructs were driven by the patatin promoter in order to confine the effects to the tuber.



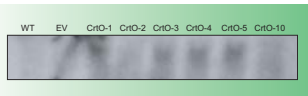
Constructs used in *Agrobacterium*-mediated potato transformation

Transgenic modification of tuber carotenoid content

Constructs have been assembled to either down-regulate potato genes encoding the carotenogenic enzymes (β-cyc and ε-cyc) or to over-express the algal *crtO* gene. Analysis of the effects of these transgenes is currently underway. Results are shown for the effects of over-expressing the algal *crtO* cDNA, encoding β-carotene ketolase in *S. tuberosum* cv Desiree. Carotenoid profiling reveals significant changes in tuber carotenoid levels. Transgenic plants contain lower levels of lutein and higher levels of carotenoid esters. More importantly, perhaps, is the presence of the commercially and nutritionally important keto-carotenoid astaxanthin in the transgenic tubers.



HPLC profile of developing tubers expressing *crtO* gene compared with control (1, neoxanthin; 2, violaxanthin; 3, antheraxanthin; 4, lutein;



Northern analysis of developing tubers expressing the *crtO* gene compared with controls

Immunoblot analysis of developing tubers expressing the *crtO* gene compared with controls

Work in Progress

Transgene stacking experiments to enhance tuber carotenoid manipulation.
Detailed analysis of transgenic potato lines with modified carotenoid content.
Microarray experiments using oligonucleotide arrays of carotenoid biosynthetic genes.

Acknowledgements

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