The role of carotenoid cleavage products in tuber apical meristem activation and deactivation processes

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Introduction

The life cycle of the potato tuber includes organogenesis, tuber development, dormancy and sprouting. This developmental sequence requires the co-ordinated control of a complex set of physiological processes and metabolic pathways and impacts directly on many economically important traits such as tuber dormancy and tuber size distribution. Thus although an understanding of meristem activation and deactivation processes is fundamental in the drive for increased tuber quality and crop productivity, there are currently gaps in our knowledge about these processes.

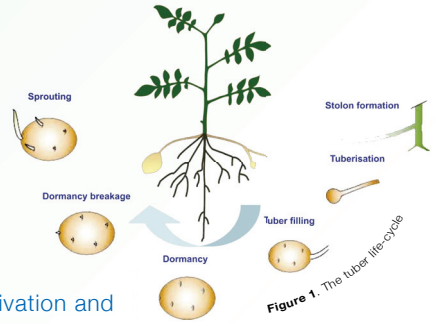

Figure 1. The tuber life-cycle

The Experiment

In the search for genes underpinning quality traits in potato a valuable resource is *S. tuberosum* group Phureja, differentiated from *S. tuberosum* group Tuberosum on the basis of a number of important tuber quality traits such as flavour, texture, colour and reduced tuber dormancy. A microarray approach was used to identify gene expression differences between the Phureja and Tuberosum types. SCRI is a partner in the Potato Oligo Chip Initiative. The POCI has produced a 60-mer oligo microarray chip on the Agilent platform. Based on EST databases > 230.000 ESTs Unigene set of 44.000 genes 42034 potato oligos on Array. This oligochip promises to be the most robust chip ever developed for the study of potato gene function. A comparison of gene expression profiles between two Tuberosum and two Phureja cultivars identified 309 genes consistently up-regulated and 555 genes consistently down-regulated in Phureja tubers compared with Tuberosum.

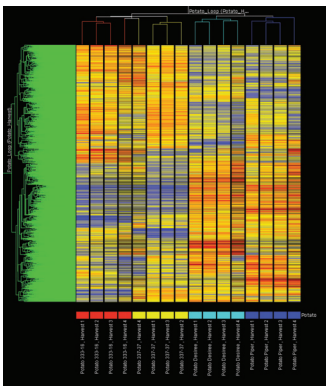

Figure 2. Statistically significant genes showing groupings of the 4 genotypes

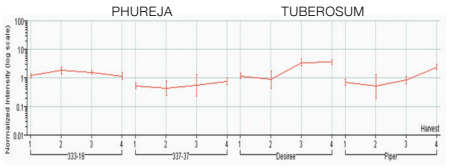

Figure 3. Gene expression profile of carotenoid cleavage dioxygenase 4 at 4 stages of tuber development.

Amongst the differentially expressed genes is carotenoid cleavage dioxygenase 4 (*CCD4*), more highly expressed in Phureja tubers at later developmental stages.

A transgenic approach was used to investigate the function of the *CCD4* gene.

Two constructs were prepared for potato transformation – an RNAi construct driven by a constitutive 35S CaMV promoter and an antisense construct driven by the tuber specific patatin promoter.

Agrobacterium-mediated transformation was used to generate transgenic lines. 32 independent RNAi lines and 33 antisense lines were generated.

Figure 4.

In the first generation transgenics severe phenotypic effects were observed. The phenotype was characterised by early sprouting during tuber development or by the formation of chain tubers (Figure 4). These effects were most pronounced in the RNAi lines (20 out of 33) although several of the antisense (but none of the 7 empty vector control lines or wild type plants, grown in the same experiment) exhibited the phenotype (Table 1).

Table 1.

	Number of lines	Lines with sprouts present	Lines with chain tubers present	Both sprouts and chain tubers present
CCD4 RNAi	33	20	17	12
CCD4 AS	32	4	4	1
WT	7	0	0	0
EV	7	0	0	0

In fact this phenotype closely resembles the heat sprouting phenotype characteristically seen when plants are exposed to temperatures in excess of 30°C. However the phenotype is also observed in microtubers grown *in vitro* under standard conditions (Figure 5).

Figure 5.

Additionally, tuber carotenoid content was elevated in the CCD4 RNAi lines (Figure 6).

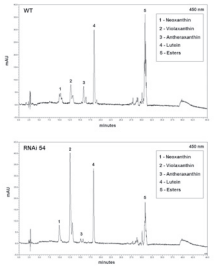

Figure 6. Tubers from RNAi lines contain up to 3-fold higher carotenoid levels, with particularly high levels of violaxanthin.

Microarray analysis using the POCI array demonstrates that there is no "off-target" silencing of other members of the carotenoid cleavage dioxygenase family (Figure 7).

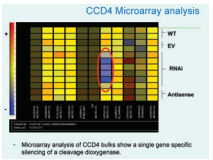

Figure 7.

Work in progress

Several questions arise from these observations:

1. Is the CCD4 gene product part of the heat sensing mechanism?
2. What is the apocarotenoid produced by the CCD4 reaction *in vivo*?
3. How is the CCD4 gene involved in the regulation of tuber meristem activation?

Acknowledgement

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