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

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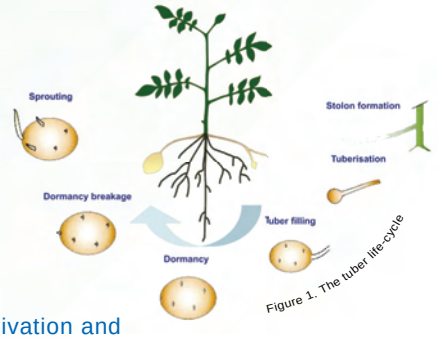


Figure 1. The tuber life-cycle

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.

The Experiment

Contrasting potato genotypes

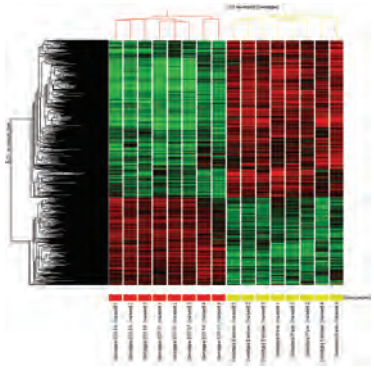
In the search for genes underpinning quality traits in potato, a valuable germplasm resource is *Solanum tuberosum* group Phureja, differentiated from *Solanum tuberosum* group Tuberosum on the basis of a number of important tuber quality traits such as flavour, texture, colour and reduced tuber dormancy.

Microarray development

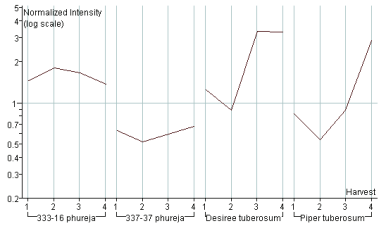
A microarray approach was used to identify gene expression differences between Phureja and Tuberosum types. SCRI is a partner in the Potato Oligo Chip Initiative (POCI) which has produced a 60-mer oligo microarray chip using the Agilent custom platform. Based on 230,000 Expressed Sequence Tags (ESTs) a unigene set of 42,000 genes are represented on the array. This microarray is the most robust chip developed for the study of potato gene function.

Gene expression analysis

A comparison of gene expression profiles between two Tuberosum and two Phureja genotypes using POCI arrays with field-grown material identified 300 genes consistently up-regulated and 550 genes consistently down-regulated in Phureja tubers compared with Tuberosum.



Amongst the significant differentially expressed genes is *CCD4*, which encodes a carotenoid cleavage dioxygenase, found to be more highly expressed in Tuberosum type tubers at later developmental stages.



CCDs break down carotenoids to apocarotenoids, including phytohormones, volatiles and other signalling molecules, involved in a wide range of important mechanisms in plants.

Development of transgenic CCD4 potatoes

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 independent RNAi (32 lines) and antisense (33 lines) *CCD4* transgenic lines.

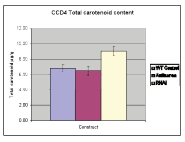
	Number of lines	Lines with sprouts present	Lines with chain tubers present	Both sprouts and chain tubers present
CCD4 RNAi	33	20	17	12
CCD4 AS	32	4	4	1
WT	7	0	0	0
EV	7	0	0	0

Phenotypes of CCD4 lines

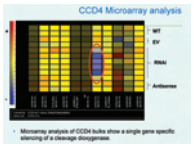
First generation transgenics showed severe phenotypic effects characterised by early sprouting during tuber development or by the formation of chain tubers. These effects were most pronounced in the RNAi lines, although several of the antisense lines also exhibited the phenotype.

This phenotype closely resembles the heat sprouting character seen when plants are exposed to temperatures in excess of 30oC and is also observed in microtubers grown under standard in vitro conditions.

Metabolite and gene expression analysis



Tuber carotenoid content was found to be significantly elevated in the CCD4 RNAi lines.



Additional microarray analysis of transgenic *CCD4* lines using the POCI array demonstrated that there was no “off-target” silencing of other members of the carotenoid cleavage dioxygenase family.

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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