Determining the Genetic Control of Carotenoids in Potato Tubers.

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Carotenoids are a diverse group of over 700 biologically active compounds present in all major plant taxa, bacteria, algae and fungi. In plants, carotenoids absorb light energy for use in photosynthesis, protect chlorophyl from photooxidative damage and are precursors for the plant hormone abscisic acid. The antioxidant properties of carotenoids provide a variety of health benefits in humans such as protection against prostate cancer and age related macular degeneration. However the most beneficial role in human health is the provitamin A activity of the β-ring carotenoids. Humans are unable to synthesise carotenoids *de novo* and therefore rely on dietary intake as a source of these important micronutrients.

Tuber flesh colour in potato ranges from white to deep yellow and is directly linked to the presence of carotenoids. The QTL for flesh colour maps to chromosome 3 in potato and is designated the *Y* locus. The aim of this project is to identify the key genes and control mechanisms influencing tuber carotenoid content using a combined genetic and molecular approach.

Phenotyping Phureja F1 mapping populations.

Figure 1. 01H15 and 03TR2 parental tubers.

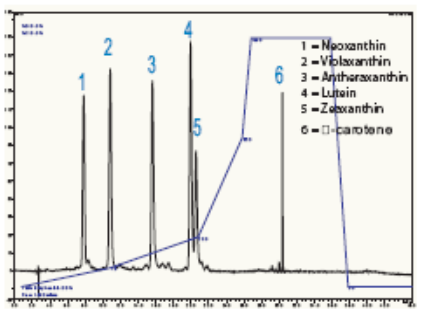


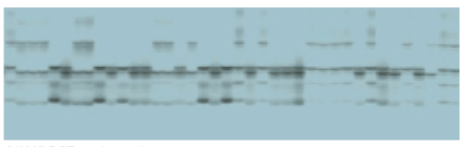
Figure 2. HPLC trace from carotenoid standards.

Two F1 diploid populations 01H15 and 03TR2 from *Solanum tuberosum* group Phureja segregating for tuber flesh colour were selected for this study (Figure 1). Population 01H15 comprises of 106 progeny and segregates 3:1 for deep yellow to pale yellow flesh. The second population, 03TR2 comprises of 177 progeny and segregates 1:1 for deep yellow to pale yellow tubers. All progeny have been extracted for total carotenoids and analysed using reverse phase chromatography over two growing seasons.

Identifying Candidate Genes.

Flesh colour scores at harvest were mapped to chromosome 3 alongside SSR and AFLP markers (Figure 3).

Two candidate genes are present on tomato chromosome 3 (figure 4), a phytoene synthase (PSY1) and a β-carotene hydroxylase (crtR-b2).



01H15 SSR marker gel.

Figure 3. 01H15 chromosome 3 linkage group.

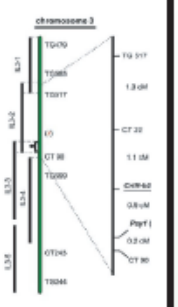


Figure 4. Tomato chromosome 3 (Thorup *et al* 2000).

Preliminary Microarray study.

Two yellow fleshed Phureja clones DB333/18 and DB333/37 were compared to two pale fleshed Tuberosum, Desiree and Maris Piper using the 44K POCI microarray. Samples were harvested over four developmental stages and the RNA prepared from freeze dried tubers.

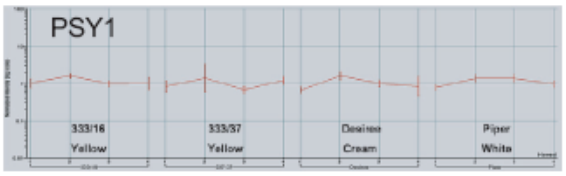


Figure 5. PSY1 expression levels in 333/16, 333/37 Desire and Maris Piper.

PSY1 has previously been shown to be a rate limiting step in carotenoid biosynthesis (Hirschberg, 2001). However there was no significant differences in expression between the yellow fleshed Phureja and the pale fleshed Tuberosum (Figure 5).

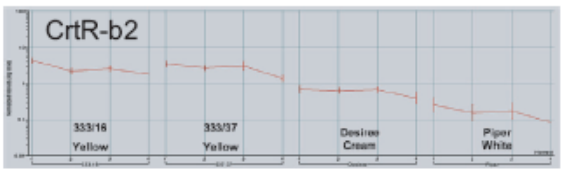


Figure 6. CrtR-b2 expression levels in 333/16, 333/37 Desiree and Maris Piper.

CrtR-b2 expression on the other hand was significantly higher in the yellow fleshed Phureja compared to the pale fleshed Tuberosum (Figure 6).

CrtR-b2 Assy

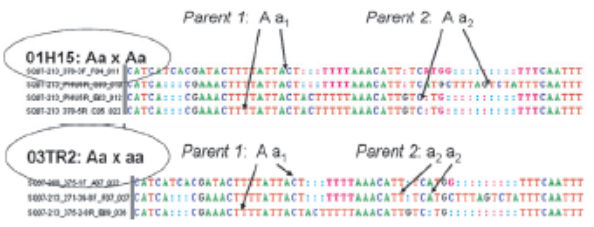


Figure 13. CrtR-b2 sequence segments from parents of 01H15 and 03TR2.

Analysis of parental crtB-2 sequences revealed that three haplotypes were present in both populations each with various differences (Figure 13).

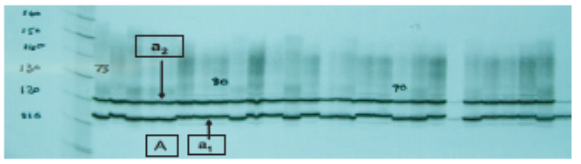


Figure 14. CrtR-b2 assay on 03TR2 progeny 73-99

03TR2 Sample	Colour Score	Total carotenoid u/g	CrtRb2 - Aa2
90	5	28.38	X
91	5	25.30	X
92	3	6.25	
93	5	29.38	X
94	4	26.29	X
95	3	8.05	
96	3	11.24	
97	3	7.75	
98	5	20.36	X
99	2	6.39	
100	3	12.17	
101	5	23.43	X
102	3	11.50	
103	5	33.83	X

Figure 15. 03TR2 phenotypic data samples 90-103.

Using the size differences between the various crtR-b2 haplotypes an assay was developed to screen all the individuals in both populations (Figure 14). When compared with the phenotypic data (Figure 15) certain crtR-b2 haplotypes matched high carotenoid samples.

Quantitative Real Time PCR.

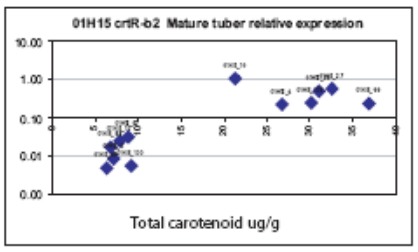


Figure 11. 01H15 crtR-b2 expression in 12 progeny.

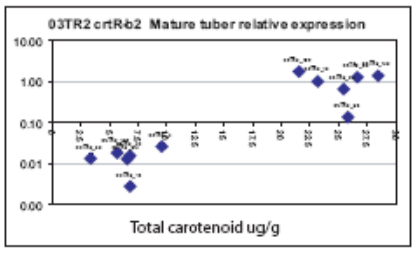


Figure 12. 03TR2 crtR-b2 expression in 12 progeny.

To confirm the results of the microarray six yellow and six pale progeny from populations 01H15 and 03TR2 were analysed for relative expression using the Roche Universal Probe Library.

The results obtained show that crtR-b2 expression in yellow fleshed progeny is signficantly higher than pale fleshed progeny (Figures 11 and 12).

Carotenoid Cleavage Dioxygenase 4 (CCD4)

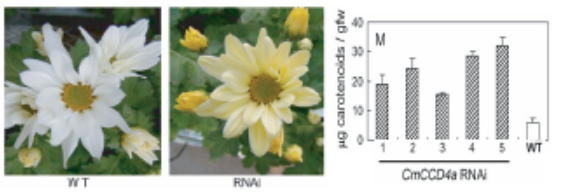


Figure 16. WT and RNAi silenced CCD4 Chrysanthemum plants with total carotenoid measurements.

Responible for the degradation of yellow carotenoids to colourless in Chrysanthemum petals a process which can be reversed using RNAi silencing (Ohimya *et al 2006.* Figure 16).
Is the same thing happening in potato tubers?

Two CCD4 silencing constructs, one using the tuber specific patatin promoter the other a constitutive RNAi silencing vector have been transformed in the cream fleshed cultivar Desiree to investigate the effect on carotenoid content in the tuber (Figure 17).

Figure 17. RNAi CCD4 transgenic currently growing in the glasshouse.