Isoprenoid metabolic networks in potato tubers

M.A. Taylor, L.J.M. Ducreux, W.L. Morris, P.E. Hedley & S. Millam

Isoprenoid biosynthetic pathways provide a wide-range of metabolites that are essential for plant development and storage organ food quality. Examples include the carotenoids which act as photosynthetic pigments and are important micronutrients in storage organs; sterols, essential for membrane function; tocopherols and tocotrienols (vitamin E); chlorophylls that contain a C20 isoprenoid side-chain; the isoprenoid derived phytohormones, gibberellins, brassinosteroids and abscisic acid; and monoterpenes, sesquiterpenes and diterpenes involved in plant defence, aroma and flavour. An understanding of how isoprenoid metabolic networks are regulated is fundamental in the drive to produce plant products of improved quality containing enhanced levels of health-promoting phytochemicals or to manipulate plant developmental processes that are regulated by the levels of the isoprenoid plant hormones.

There has been a rapid escalation in our knowledge of the isoprenoid biosynthetic pathways, particularly at the molecular level. Isoprenoids are synthesised from the 5-carbon intermediates isopentenyl diphosphate and dimethylallyl diphosphate. Although many of the genes encoding the structural enzymes involved in isoprenoid biosynthesis have been cloned, so far only limited data about their integrated regulation have been obtained using classical genetic and biochemical approaches or more recently using a microarray approach in *Arabidopsis*. Additionally, in view of the pace of progress in this area, it is perhaps surprising that the factors that regulate isoprenoid pathways remain to be discovered. This is in contrast to other important metabolic networks such as phenylpropanoid metabolism, where a number of regulatory genes and factors have been described.

Using a metabolic engineering approach, we have attempted to perturb aspects of isoprenoid metabolism in potato tubers. Our aims are to determine the extent to which it is possible to enhance the accumulation of nutritionally important isoprenoids such as carotenoids, and to learn more about the integrated regulation of isoprenoid metabolism. In one example we have produced transgenic potato plants expressing an *Erwinia uredovora crtB* gene encoding phytoene synthase, specifically in the tuber of *Solanum tuberosum* L. cultivar Désirée, which normally produces tubers of low carotenoid content (8.6 µg carotenoid g^{-1} DW) and also in *Solanum phureja* L. DB337 (tuber carotenoid content typically 20 µg carotenoid g^{-1} DW). In developing tubers of transgenic *crtB* Désirée lines, carotenoid levels reached 35 µg carotenoid g^{-1} DW and the balance of carotenoids changed radically compared with controls: β-carotene levels in the transgenic tubers reached *c.* 11 µg g^{-1} DW, whereas control tubers contained negligible amounts and lutein accumulated to a level of 12 µg g^{-1} DW, 20-fold higher than controls (Fig. 1). Following the development of a novel transformation protocol for potato diploids, the *crtB* gene was also transformed into *S. phureja* DB337, and a large increase in total carotenoid content (to 78 µg carotenoid g^{-1} DW) was measured in the most affected transgenic line. In these tubers, the major carotenoids were violaxanthin, lutein, antheraxanthin and β-carotene. No increases in expression levels of the major carotenoid biosynthetic genes could be detected in the transgenic tubers, despite the large increase in carotenoid accumulation. Thus by over-expressing the *crtB* gene, we can produce potato tubers containing carotenoids not normally found at significant levels in potato germplasm (β-carotene). This finding is of particular importance as β-carotene is the major provitamin A carotenoid and the levels found in tubers from the best transgenic lines could contribute significantly to the dietary vitamin A requirement. On a dry weight basis, the β-carotene content of *crtB* tubers is up to 7-fold greater than that of the highest published value for 'Golden rice'.

Having successfully perturbed isoprenoid metabolism, we applied a combined isoprenoid and transcript profiling approach to gain further insights into the regulatory mechanisms that control isoprenoid levels. Microarray analysis was used to identify a number of genes that were consistently up or down-regulated in transgenic *crtB* tubers. We are currently using a gene silencing approach to investigate further the roles of these candidate genes. In collaboration with the Cell-to-Cell Communication Programme we previously demonstrated that a modified potato virus X vector could be used to silence gene expression in potato leaves and tubers and we are exploiting this approach in our study of the candidate genes revealed by microarray analysis. Over-expression of other carotenoid biosyn-

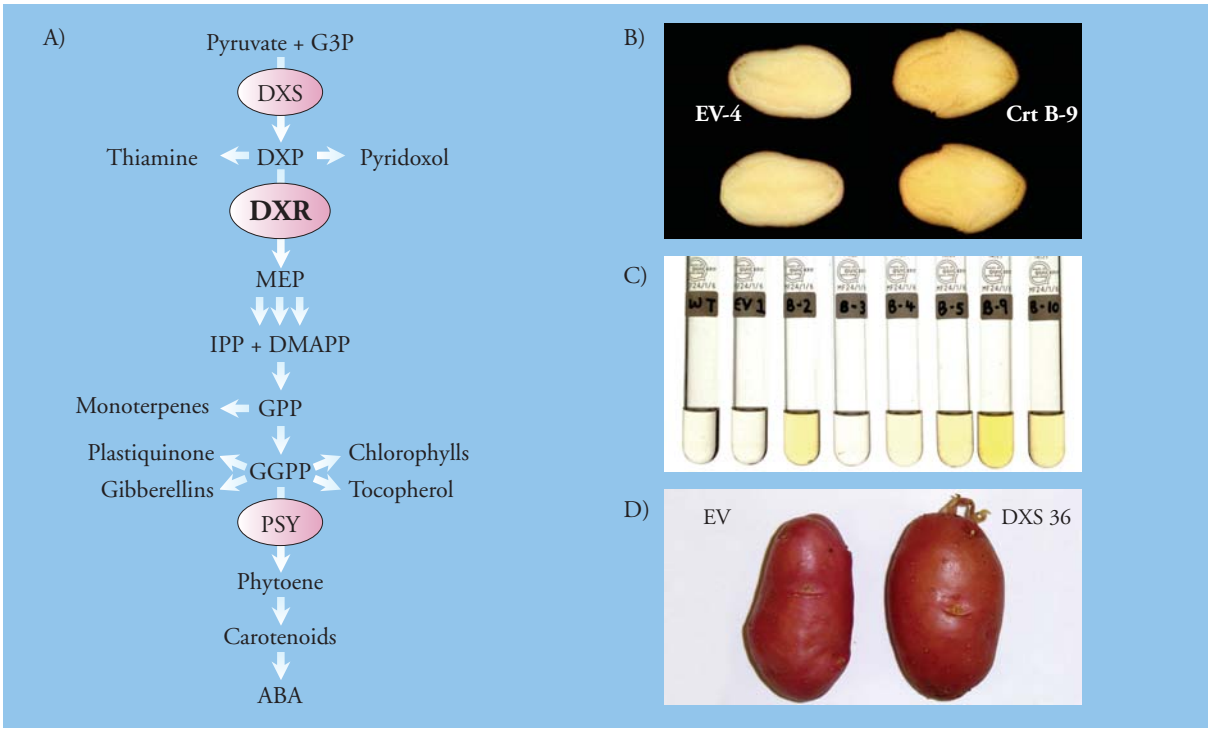

Figure 1 A) The biochemical pathway of isoprenoid metabolism in the plastid. B) Mature tubers of *S. tuberosum* cv. Désirée expressing the *Erwinia uredovora crtB* gene (crtB-9) compared with controls (Ev-4). C) Solvent extracts of carotenoids from transgenic tubers expressing *crtB*. D) Transgenic tubers at harvest, expressing a bacterial 1-deoxy-D-xylulose 5-phosphate synthase gene (DXS36) compared with control (EV).

thetic genes has also led to the accumulation of carotenoids not normally found in potato tubers. For example, tuber-specific expression of an algal ketolase gene (*crtO*) leads to the accumulation of the carotenoid astaxanthin, increasingly being marketed as a neutraceutical because of an array of health benefits associated with its consumption. We are currently exploring techniques for introducing several transgenes simultaneously into potato (co-transformation) so that we can increase levels of accumulation further.

In another example of metabolic engineering we targeted a very early reaction in the biosynthesis of plastidic isoprenoids. Some literature suggests that the first step in the reaction path to isopentenyl pyrophosphate, the acyloin condensation of glyceraldehyde-3-phosphate with pyruvate catalysed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS), limits the rate of carotenoid biosynthesis. Thus we were interested in investigating the effects of over-expressing this enzyme in the tuber plastid. We over-expressed an *E. coli* DXS gene in the plastid of the potato tuber and determined the effects on isoprenoid biosynthesis. No major changes in tuber carotenoid content were detected in developing tubers; however, on harvesting mature tubers we observed that in many of the transgenic lines (approximately 20 out of 40) there was an 'early sprouting' phenotype – many tubers were already sprouting at harvest and in some lines there was a loss of apical dominance (Fig. 1). This is a robust phenotype, the 'severity' of which correlates with the level of expression of the DXS transgene. Interestingly, the early sprouting phenotype is similar to that observed for tubers of many *S. phureja* accessions. We suspect that in these transgenics there are enhanced levels or rates of gibberellin biosynthesis, that is, the increased DXS activity results in flux being driven to isoprenoids other than the carotenoids. These transgenics thus provide an invaluable and unique opportunity to investigate aspects of isoprenoid metabolic control and provide a novel resource with a clear phenotype that impacts on aspects of the tuber life-cycle of commercial importance (tuber sprouting and dormancy).

In summary, our metabolic engineering approach has confirmed that there is considerable flexibility in the types and amounts of nutritionally important isoprenoids that can accumulate in tubers. Additionally we have indications that the control of isoprenoid metabolic flux impacts on important aspects of the tuber life-cycle.