

Genetic modification of plant metabolism for human health benefits

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Abstract

There has been considerable research progress over the past decade on elucidating biosynthetic pathways for important human health components of crops. This has enabled the use of genetic modification (GM) techniques to develop crop varieties with increased amounts of essential vitamins and minerals, and improved profiles of ‘nutraceutical’ compounds. Much of the research into vitamins and minerals has focused on generating new varieties of staple crops to improve the diet of populations in developing nations. Of particular note is the development of new rice lines with increased amounts of provitamin A and iron. Research on modifying production of nutraceuticals has generally been aimed at generating new crops for markets in the developed nations, commonly to deliver distinctive cultivars with high consumer appeal. Most progress on nutraceuticals has been made with just a few types of metabolites to date, in particular in the production of novel long-chain polyunsaturated fatty acids in oil-seed crops and to increase amounts of flavonoids and carotenoids in tomato and potato. However, given the rapid progress on elucidating plant metabolite biosynthetic pathways, wide-ranging success with metabolic engineering for levels of human health-related compounds in plants would be expected in the near future. A key aspect for future success will be better medical information to guide metabolic engineering endeavors. Although the desired levels of many vitamins are known, detailed information is lacking for most of the nutraceuticals that have attracted much interest over the past few years.

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1. Introduction

There is growing evidence that specific dietary components, the so-called ‘nutraceutical’ metabolites often found in plant-based foods, may help prevent or control particular diseases and disorders. The recent completion of the human genome sequence and associated advances in proteomics and transcriptomics has accelerated research on the development of tests to indicate an individual’s propensity towards disease or health problems. Bringing together the findings on the role of diet with these ‘omics technologies has resulted in the field of nutrigenomics [1], and added to the drive to develop plant cultivars with improved profiles of health-related metabolites. A potential route for delivering the new plant lines is to use genetic modification (GM) technolo-

Abbreviations: 1-SST, 1-sucrose:sucrose fructosyltransferase; 35SCaMV, Cauliflower Mosaic Virus 35S promoter; 6G-FFT, fructan:fructan 6-glucose-fructosyltransferase; ARA, arachidonic acid; bHLH, basic helix-loop-helix; β -LYC, β -lycopene cyclase; CDS, carotene desaturase; CHS, chalcone synthase; CHI, chalcone isomerase; CLA, conjugated linoleic acids; DFR, dihydroflavonol 4-reductase; DHA, docosahexaenoic acid; DW, dry weight; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; EPA, eicosapentaenoic acid; FW, fresh weight; GCH1, GTP cyclohydrolase-1; GM, genetic modification; IFS, isoflavone synthase; LC-PUFAs, long-chain polyunsaturated fatty acids; PDS, phytoene desaturase; PSY, phytoene synthase; RDA, recommended daily allowance; RNAi, RNA interference; STS, stilbene synthase; TDC, tryptophan decarboxylase; TF, transcription factor

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gies to accelerate the breeding process. In the decade or so since their introduction, GM crops have had a major impact on agriculture in several countries. The new traits introduced into commercial cultivars to date have been for herbicide tolerance or improved pest and disease resistance, primarily for use in the agriculture systems of the developed world. However, there has also been considerable research progress on elucidating biosynthetic pathways for important human health components of crops, and there are already GM crop lines available with increased amounts of essential vitamins or proposed nutraceuticals.

The new GM crop lines that have been developed with improved human health characters have yet to be commercialised, but there are several varieties that may be released for agricultural use in the next 5–10 years. Much of the vitamin-related research to date has focused on improving the diet of populations in developing nations, where malnutrition may occur through lack of variety in the diet. Rural diets in many developing countries have traditionally been based on cereals, legumes, and starchy roots or tubers, which generally have lower micronutrient content than diets that include flesh foods such as meat, poultry and fish. In particular, some of the key cereals, such as rice, that provide the major source of nutrition for some populations, are lacking in specific vitamins and minerals essential to health. Research on modifying production of nutraceutical compounds for new functional foods has generally been aimed at generating new crops for markets in the developed nations, commonly to deliver distinctive cultivars with high consumer appeal.

In this short review, no attempt is made to make a comprehensive list of all the reports on metabolic engineering of plants for improved human health characteristics. Rather, examples of recent advances are given, with discussion of some of the issues to be resolved. The review is focused principally on plant pathways for small metabolites. However, it is important to note that there is also considerable progress on GM for improved protein production in crops. This is for both quantity and quality, such as introduction of proteins with an improved amino acid balance [2] and reduction of levels of potential allergens (e.g. [3]). Emphasis is given to data for crop plants such as rice (*Oryza sativa*), tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*), rather than extensive discussion of experiments in model systems.

2. Metabolic engineering approaches for modification of metabolite biosynthesis

Rational GM approaches to metabolic engineering of a biosynthetic pathway require knowledge of the pro-

duction and accumulation of the metabolites of interest, the availability of DNA sequences encoding appropriate biosynthetic enzymes or regulatory factors, and gene transfer methods for the target species. Given a sufficient knowledge of the target system, predictive metabolic engineering approaches may be applied, in which data from metabolomics, transcriptomics and proteomics are used to identify key targets, such as flux control points or regulatory proteins [4]. However, at present, the required information and tools are available only for a few pathways and crops. For most pathways there is incomplete knowledge of the genes involved, key flux points, regulatory factors and the impact of cellular compartmentalisation or metabolic channeling. Furthermore, there may be unknown genetic variations in the recipient germplasm that impact on the efficacy of the transgene, for example mutations in biosynthetic genes of the target pathway. Thus, in many cases, a reiterative ‘trial and error’ approach has been used to try and identify the required combination of transgenes.

Two basic approaches to modifying a biosynthetic pathway to increase amounts of desirable compounds may be identified – manipulation of pathway flux, or introduction of novel biosynthetic activities from other organisms. The methods for increasing, preventing or redirecting flux into or within the pathway include: increasing levels of a rate-limiting biosynthetic enzyme, inhibition of the activity of a gene that competes for a limited substrate supply, and up- or down-regulation of the pathway using regulatory factors. For reducing production of undesirable compounds the well-proven approach is to inhibit gene activity for one of the biosynthetic enzymes. RNA interference (RNAi) is an effective and reliable approach for preventing enzyme production, with examples of better performance than using antisense or sense-inhibition constructs [5].

Results for the flavonoid pathway demonstrate that all of the above-mentioned approaches can be applied successfully to modify plant metabolite production (reviewed in [6]). This includes not only the use of transgenes for biosynthetic enzymes but also extensive examples of the power of transcription factor (TF) transgenes to up-regulate the pathway. For example, at least eight biosynthetic genes leading to anthocyanin production were greatly up-regulated by a single MYB transgene in petunia (*Petunia* sp.) [7], and over-expression of an AtMYB12 transgene in arabidopsis (*Arabidopsis thaliana*) caused the specific up-regulation of four biosynthetic genes for flavonol production [8]. There is also growing evidence that TFs are commonly a major determinate of the observed variation in plant secondary metabolite biosynthesis [9], making them good

candidate genes for molecular marker programmes for marker assisted breeding. Thus, it would be expected that TF transgenes will be widely used in future GM approaches. However, the flavonoid pathway is one of the best-characterised of plant biosynthetic pathways. Appropriate TFs have yet to be identified for most metabolite pathways, although rapid progress on assignment of TF gene function is being made in various genomics programmes. An alternative is the construction of synthetic TFs, and this approach has been used to increase tocopherol amounts by modifying a zinc-finger TF to introduce a DNA-recognition motif that recognised the promoter of a γ -tocopherol methyltransferase gene [10].

3. Metabolic engineering approaches for vitamin biosynthesis

Table 1 lists some of the GM approaches for modifying production of essential vitamins. It is not an exhaustive list but provides an overview of the pathways and approaches targeted, with an emphasis on more recent literature. It can be seen from the table that there has been notable success for at least four major vitamins A, B (folate), C and E.

Of particular note is the substantial progress towards generating 'Golden Rice' cultivars that could make a significant contribution of provitamin A to the diet of large population groups. Vitamin A deficiency is thought to affect populations in at least 26 countries [80]. Provitamin A is the term for the plant carotenoid pigments that are converted to retinol (vitamin A) in animals through their degradation in an active and regulated process (e.g. [81]). All-*trans*- β -carotene has the most provitamin A activity, and it is this carotenoid that has been the prime focus of the molecular breeding programme. No rice germplasm capable of synthesising carotenoids in the endosperm has been identified for use in conventional breeding programmes, so GM is the only current option for producing rice cultivars high in carotenoids. The direct engineering of retinol biosynthesis into plants has not been targeted, one reason being that retinol supplementation may cause side-effects at amounts greater than 5 \times the RDA, while amounts of provitamin A are safe at least up to 100 \times the RDA [82]. Recently developed versions of Golden Rice have up to 37 $\mu\text{g g}^{-1}$ DW total carotenoids in the endosperm (of which 31 $\mu\text{g g}^{-1}$ DW is β -carotene) [13]. The developers estimate, using conservative figures, that at these levels 72 g of dry, polished Golden Rice could provide 50% of a child's RDA of vitamin A. More accurate figures will require further efficacy data, for example on the bioavailability from actual

diets, but further recent theoretical impact assessments [83] also support Golden Rice as a useful, cost-effective component in programmes to address malnutrition. Al-Babili and Beyer [84] recently provided an excellent overview of the production of Golden Rice, trials of the crop in rice-growing nations, and the progress towards its deregulation.

Vitamin E is a collective term for a group of lipid-soluble antioxidants of photosynthetic organisms called tocochromanols, comprised of tocopherols and tocotrienols [85,86]. There are four types of naturally occurring tocopherols and tocotrienols, α , β , δ and γ , that differ in the number and position of methyl groups on the aromatic ring. Plants and other photosynthetic organisms are the usual source of tocopherols in the diet. The amount of tocopherols in food crops varies greatly, for example from 0.7 $\mu\text{g g}^{-1}$ FW in potato tubers up to 2700 $\mu\text{g g}^{-1}$ oil in wheat germ oil [86]. Knowledge about the biosynthetic pathway for tocochromanols has advanced rapidly over the past 10 years [85,86]. The identification of genes for the biosynthetic steps has enabled a range of successful GM approaches for increasing vitamin E activity in plant sources (Table 1). These have included both increasing total tocochromanol amounts and altering the specific forms produced. Examples of the latter include increasing amounts of tocotrienols (which are more commonly produced in monocots) as opposed to tocopherols [27] and promoting production of α -tocopherol, which has the highest vitamin E activity in mammals [10,29]. The recent comprehensive study by Karunanandaa et al. [30] combined these various approaches to produce seeds of oil crops with a tocochromanol content of up to 4800 $\mu\text{g g}^{-1}$ oil.

Folates (vitamin B₉) are involved in a range of metabolic processes in humans. Inadequate amounts of folate in the diet can trigger a range of health issues, such as increased risk of cardiovascular disease and cancer. Folate is particularly important in pregnancy, for prevention of megaloblastic anemia and birth defects. Neural tube defects affect approximately one of every 1000 live births in the United States, and this rate is even higher in other nations [35]. Plants are a primary source of dietary folates, but the folate content of many staple crops is quite low. To date, the research focus has generally been on gaining better understanding of the biosynthesis pathway in model systems such as *Arabidopsis*, and there are only a few metabolic engineering examples for crop species. One of these is for tomato, in which fruit-specific overexpression of a feedback-insensitive GTP cyclohydrolase-1 (GCH1) resulted in a modest increase (2-fold) of fruit folate amounts [36]. GCH1 is the first enzyme in the biosynthesis of one precursor

Table 1

Selected examples of the metabolic engineering of plant metabolism for improving human health-related characters. Emphasis is given to recent studies on crop species

Target compound	Species	Approach	Impact (general comment only)	References
Carotenoids (including provitamin A)	<i>Brassica napus</i>	Seed-specific overexpression of bacterial geranylgeranyl diphosphate synthase, phytoene synthase (PSY) and phytoene desaturase (PDS) and plant or bacterial β -lycopene cyclase (β -LYC)	50-fold increase in seed carotenoid levels (principally carotenes), a orange colour imparted and altered α - to β -carotene ratio	[11]
	<i>Oryza sativa</i>	Endosperm-specific overexpression of daffodil (<i>Narcissus pseudonarcissus</i>) PSY and <i>Erwinia uredovora</i> carotene desaturase (CDS)	Development of 'Golden Rice' Indica and Japonica cultivars amenable to deregulation, with carotenoid levels ranging from 0.4 to 1.2 $\mu\text{g g}^{-1}$ dry seed	[12]
	<i>Oryza sativa</i>	Endosperm-specific overexpression of maize PSY and <i>E. uredovora</i> CDS	23-fold higher total carotenoid content than the original Japonica 'Golden Rice', with β -carotene at up to 31 $\mu\text{g g}^{-1}$ dry seed	[13]
	<i>Nicotiana tabacum</i> ,	Overexpression of carotenoid-4,4'-oxygenase and	Formation of ketolated carotenoids in leaves of tobacco (at up to	[14]
	<i>Solanum lycopersicum</i>	carotenoid-3,3'-hydroxylase from the marine bacteria <i>Paracoccus</i> sp.	800 $\mu\text{g g}^{-1}$ DW), and at low levels in leaves and fruit of tomato	[15]
	<i>Solanum lycopersicum</i>	Overexpression of <i>E. uredovora</i> PSY	2 to 4-fold increase in fruit carotenoid levels	[16]
	<i>Solanum lycopersicum</i>	Overexpression of tomato PSY	Increased carotenoid production in fruit and other tissues, pleiotropic effects	[17,18]
	<i>Solanum lycopersicum</i>	Overexpression of <i>E. uredovora</i> PSY	Increased accumulation of β -carotene and its derivatives, overall decrease in carotenoid content	[19]
	<i>Solanum lycopersicum</i>	Fruit-specific overexpression of β -LYC and β -carotene hydroxylase	Significant increase in levels of β -carotene, β -cryptoxanthin and zeaxanthin in fruit	[20,21]
	<i>Solanum lycopersicum</i>	Overexpression of β -LYC	Up to 7-fold increase in β -carotene levels in fruit, colour change from red to orange	[20]
	<i>Solanum lycopersicum</i>	Inhibition of gene activity for β -LYC	Loss of β -carotene, small increase in lycopene content of fruit	[22]
	<i>Solanum lycopersicum</i>	Overexpression (35SCaMV or fibrillin promoter) of <i>E. coli</i> 1-deoxy-D-xylulose-5-phosphate synthase (DXS)	Carotenoid levels in <i>fibrillin:DXS</i> fruit increased up to 1.6-fold	[23]
	<i>Solanum tuberosum</i>	Inhibition of gene activity for zeaxanthin epoxidase	Zeaxanthin produced	[24]
	<i>Solanum phureja</i> , <i>Solanum tuberosum</i>	Overexpression of <i>E. uredovora</i> PSY	Carotenoid content increased from 5.6 to 35 $\mu\text{g g}^{-1}$ DW in tubers of <i>S. tuberosum</i> , and from 20 to 78 $\mu\text{g g}^{-1}$ DW in tubers of <i>S. phureja</i> . Levels of β -carotene and lutein were particularly increased	[25]
	<i>Solanum phureja</i> , <i>Solanum tuberosum</i>	Tuber-specific overexpression of β -carotene ketolase gene from the unicellular green alga <i>Haematococcus pluvialis</i>	Formation of ketolated carotenoids in tubers, specifically ketolutein and astaxanthin, at up to 14 $\mu\text{g g}^{-1}$ DW	[26]
<i>Solanum tuberosum</i>	Inhibition of gene activity for ϵ -lycopene cyclase	Total carotenoid levels in tubers increased up to 2.5-fold and β -carotene levels increased up to 14-fold	[27]	
Tocopherols	<i>Arabidopsis thaliana</i> , <i>Zea mays</i>	Overexpression of barley (<i>Hordeum vulgare</i>) homogentisate geranylgeranyltransferase	Tocochromanol content increased up to 15-fold in arabidopsis leaves and up to 6-fold in maize seed. Tocotrienol biosynthesis conferred to species/tissues that lacked it	[10]
	<i>Arabidopsis thaliana</i>	Expression of a synthetic zinc finger transcription factor gene to increase γ -tocopherol methyltransferase gene expression	Reduction in δ -tocopherol levels and an increase in α -tocopherol levels	[28]
	<i>Brassica napus</i>	Overexpression of tocopherol cyclases from arabidopsis and maize	Increase in total tocopherol levels in seed by up to 3.6-fold	[29]
	<i>Glycine max</i>	Overexpression of 2-methyl-6-phytylbenzoquinol methyltransferase and γ -tocopherol methyltransferase	Reduction in δ -tocopherol levels and an up to 8-fold increase in α -tocopherol levels, resulting in up to a 5-fold increase in vitamin E activity in seed	[30]
	<i>Glycine max</i>	Lines with seed-specific overexpression of arabidopsis homogentisate dioxygenase and GGDP dehydrogenase and <i>Erwinia herbicola</i> chorismate mutase/prephenate dehydrogenase were crossed to the lines of Van Eenennaam et al. [29]	Greatly increased tocochromanol levels in seed, including high proportions of α -tocopherol and α -tocotrienol, resulting in an 11-fold increase in total vitamin E activity	

Table 1 (Continued)

Target compound	Species	Approach	Impact (general comment only)	References
Vitamin C	<i>Arabidopsis thaliana</i>	Overexpression of NADPH-dependent D-galacturonate reductase	Vitamin C content increased 2 to 3-fold	[31]
	<i>Lactuca sativa</i> , <i>Nicotiana tabacum</i>	Overexpression of rat L-gulonolactone oxidase	Vitamin C content increased up to 7-fold	[32]
	<i>Nicotiana tabacum</i> , <i>Zea mays</i>	Overexpression of dehydroascorbate reductase to increased ascorbate recycling	Vitamin C content increased 2 to 4-fold in maize and tobacco leaves and maize kernels	[33,34]
Folate	<i>Arabidopsis thaliana</i>	Overexpression of a feedback-insensitive bacterial GTP cyclohydrolase-1	Increase in levels of pterins (1250-fold) and folates (2 to 4-fold)	[35]
	<i>Solanum lycopersicum</i>	Fruit-specific overexpression of a feedback-insensitive synthetic GTP cyclohydrolase-1	Increase in levels of pterins (3 to 140-fold) and folates (2-fold) in fruit. Addition of the precursor <i>p</i> -aminobenzoate enabled 10-fold increases in folate levels	[36]
Iron	<i>Lactuca sativa</i>	Overexpression of ferritin	Iron levels increased in vegetative tissues by up to 1.7-fold	[37]
	<i>Oryza sativa</i> , <i>Triticum aestivum</i>	Overexpression of ferritin	Increased iron levels in vegetative tissues but not the grain	[38]
	<i>Oryza sativa</i>	Seed-specific overexpression of ferritin	Increase in iron levels of up to 3 to 4-fold in milled grain. Increases in zinc content also observed	[39,40]
	<i>Oryza sativa</i>	Increased bioavailability through raising cysteine residue levels using a metallothionein-like sequence	Significant increase (ca. 7-fold) in cysteine content in seed soluble protein	[41]
	<i>Oryza sativa</i>	Reduction of phytic acid levels by overexpression of a thermo-tolerant phytase sequence from <i>Aspergillus fumigatus</i>	Levels of the phytase in the seeds increased about 130-fold. The expected thermo-tolerance to cooking was not observed	[42]
Selenium	<i>Arabidopsis thaliana</i> , <i>Brassica juncea</i>	Overexpression of selenocysteine methyltransferase from the selenium hyperaccumulator <i>Astragalus bisulcatus</i>	Selenium levels increased ca. 2-fold	[43]
	<i>Arabidopsis thaliana</i>	Overexpression of a chloroplastic NifS-like protein for release of Su and Sel from amino acids	Selenium levels increased ca. 2-fold	[44]
Zinc	<i>Hordeum vulgare</i>	Overexpression (ubiquitin promoter) of an arabidopsis zinc transporter (AtZIP1)	Increased zinc and iron content in seeds of plants grown under some soil conditions	[45]
LC-PUFAs	<i>Brassica juncea</i>	Generation of a series of transgenics with different gene constructs, including the use of a construct for nine different fatty acid biosynthetic enzymes	Production of arachidonic acid (ARA) and eicosapentaenoic acid (EPA) as up to 25% and 15%, respectively, of total seed fatty acids. Production of docosahexaenoic acid (DHA) in seed at relatively low levels	[46]
	<i>Glycine max</i>	Seed-specific overexpression of three fatty acid biosynthetic enzymes from <i>Mortierella alpina</i> and down-regulation of the endogenous $\Delta 15$ -desaturase	Production of non-host fatty acids up to 8.4% of total seed fatty acids. These were γ -linolenic acid, eicosa-8,11-dienoic acid, dihomo- γ -linolenic acid and ARA	[47]
	<i>Glycine max</i>	Overexpression of six fatty acid biosynthetic enzymes from a range of source species	Production of EPA at up to 20% of seed oil, and production of DHA in somatic embryos	[48]
	<i>Linum usitatissimum</i> , <i>Nicotiana tabacum</i>	Seed-specific overexpression of $\Delta 6$ -desaturase, $\Delta 5$ -desaturase and $\Delta 6$ -elongase from a range of species	Production of ARA and EPA in seeds at up to 5% of total seed fatty acids	[49]
	<i>Oryza sativa</i>	Overexpression of a bacterial (<i>Propionibacterium acnes</i>) linoleate isomerase that can convert linoleic acid to <i>trans</i> -10, <i>cis</i> -12 conjugated linoleic acid	Production of <i>trans</i> -10, <i>cis</i> -12 conjugated linoleic acid as up to 1.3% of total seed fatty acids	[50]
Phytosterols	<i>Solanum lycopersicum</i>	Overexpression of arabidopsis 3-hydroxy-3-methylglutaryl CoA reductase	Levels of phytosterols (β -sitosterol, stigmasterol and campesterol) in fruit increased up to 2.4-fold	[22]
Chlorogenic acid	<i>Solanum lycopersicum</i>	Constructs to prevent or increase production of hydroxycinnamoyl-CoA quinate: hydroxycinnamoyl transferase	Plants with either greatly reduced or increased levels of chlorogenic acid	[51]
Flavonoids	<i>Linum usitatissimum</i>	Overexpression of chalcone synthase (CHS), chalcone isomerase (CHI), and dihydroflavonol 4-reductase (DFR)	Increase in total phenolic antioxidant levels, including slight increase (12–14%) in lignan content	[52]
	<i>Solanum lycopersicum</i>	Overexpression of CHI	Increased flavonol levels	[53]
	<i>Solanum lycopersicum</i>	Overexpression of CHI and flavone synthase	Flavones produced in fruit	[54]

	<i>Solanum lycopersicum</i>	Overexpression of CHS and polyketide reductase	6'-deoxychalcones produced in fruit	[54]
	<i>Solanum lycopersicum</i>	Overexpression of tomato ANTI1 (MYB transcription factor)	Anthocyanins increased	[55]
	<i>Solanum lycopersicum</i>	Overexpression of <i>Antirrhinum majus</i> DELILA (bHLH transcription factor)	Anthocyanins increased	[56]
	<i>Solanum lycopersicum</i>	Overexpression of maize <i>Lc</i> (bHLH)	Anthocyanins increased under high light levels	[57]
	<i>Solanum lycopersicum</i>	Overexpression of <i>Perilla frutescens</i> MYC-RP and MYC-GP (both bHLH)	Anthocyanins increased	[58]
	<i>Solanum lycopersicum</i>	Overexpression of maize C1 (MYB) and LC (bHLH)	Flavonols increased in fruit; anthocyanins increased in leaves	[59]
	<i>Solanum lycopersicum</i>	Fruit specific RNAi down-regulation of the <i>DETI</i> gene	Increased flavonoid and carotenoid levels in fruit	[60]
	<i>Solanum lycopersicum</i>	Overexpression of the photoreceptor CRYPTOCHROME2	Increased flavonoid and carotenoid levels in fruit	[61]
	<i>Solanum tuberosum</i>	Overexpression of DFR	Increased anthocyanin levels in tubers	[62]
	<i>Zea mays</i>	Overexpression of maize P1 (bHLH)	Increased anthocyanin and flavone levels	[63]
Isoflavonoids	<i>Lupinus albus</i>	Overexpression of IFS	Genistein produced	[64,65]
	<i>Glycine max</i>	Overexpression of maize C1 (MYB) and R (bHLH)	Ratio of genistein to daidzein reduced	[66]
	<i>Glycine max</i>	Overexpression of maize C1 (MYB) and R (bHLH) and inhibition of flavanone 3-hydroxylase gene activity	Isoflavonoids increased	[66]
Resveratrol	<i>Brassica napus</i>	Seed-specific overexpression of stilbene synthase (STS) and inhibition of UDP-glucose:sinapate glucosyltransferase gene activity	High levels of resveratrol glycosides produced in seed and a marked reduction in sinapate ester levels	[67]
	<i>Rehmannia glutinosa</i>	Overexpression of STS	Resveratrol aglycones and glycosides produced	[68]
	<i>Solanum lycopersicum</i>	Overexpression of STS	Resveratrol aglycones and glycosides produced in fruit	[54,69]
Carbohydrates	<i>Ficus vulgaris</i>	Overexpression of Jerusalem artichoke (<i>Helianthus tuberosus</i>) 1-sucrose:sucrose fructosyltransferase (1-SST)	Production of fructan at up to 110 $\mu\text{mol g}^{-1}$ FW in tubers	[70]
	<i>Beta vulgaris</i>	Overexpression (ubiquitin promoter) of <i>Allium cepa</i> (onion) 1-SST and fructan:fructan 6G-fructosyltransferase (6G-FFT)	Production of onion-type fructans (inulin neo-series) in tubers	[71]
	<i>Cichorium intybus</i>	Overexpression of onion 6G-FFT	Production of onion-type fructans (inulin neo-series) in leaves	[72]
	<i>Cichorium intybus</i>	Overexpression of barley sucrose:fructan 6-fructosyltransferase	Production of onion-type fructans (tetrasaccharide bifurcose) in leaves	[73]
	<i>Solanum tuberosum</i>	Overexpression of bacterial (<i>Bacillus subtilis</i> or <i>Streptococcus mutans</i>) fructosyltransferase	Production of fructans at up to 30% DW in leaves and up to 7% DW in tubers	[74]
	<i>Solanum tuberosum</i>	Overexpression of bacterial (<i>Erwinia amylovora</i>) levan synthase and inhibition of ADP-glucose pyrophosphorylase gene activity	Production of fructan (levan-type) in tubers at up to 19% DW	[75]
	<i>Solanum tuberosum</i>	Overexpression of globe artichoke (<i>Cynara scolymus</i>) 1-SST and fructan:fructan 1-fructosyltransferase	Production of inulin molecules at up to 20 $\mu\text{mol g}^{-1}$ FW in tubers, with similar structures as those found in artichoke roots	[76]
	<i>Solanum tuberosum</i>	Antisense RNA inhibition of starch-branching enzyme II activity	Increase of amylose as a proportion of starch in tubers to up to 75%	[77]
	<i>Triticum aestivum</i>	RNAi of starch-branching enzyme II activity	Increase of amylose as a proportion of starch in the grain to >70%	[78]
	Toxins/antitoxins	<i>Brassica napus</i>	RNAi inhibition of gene activity for UDP-Glc:sinapate glucosyltransferase	Levels of sinapate esters in transgenics were reduced by up to 76% in seeds of the T3 generation
<i>Manihot esculenta</i>		Inhibition of activity of two genes for a cytochrome P450 enzyme that conducts the first committed step in cyanogenic glucoside biosynthesis	Levels of the cyanogenic glucosides linamarin and lotaustralin in transgenics were reduced by 92% in tubers, and virtually eliminated from leaves	[79]

to folates, the pteridines. Feeding of another precursor, the plastid biosynthesised compound *p*-aminobenzoate, gave a 10-fold increase of folates in transgenic fruit, suggesting that up-regulation of both precursor pathways may be required. With rapid advances in the metabolic engineering of plant biosynthetic pathways, it is likely that significant progress will be made in improvement of folate amounts in crop species over the next few years. Research prospects in this field have recently been reviewed by Storozhenko et al. [87] and Finglas et al. [88].

Various GM approaches have proven successful in increasing vitamin C (ascorbic acid) amounts in plants (Table 1). Only one published study includes data for a crop plant, maize (*Zea mays*). Chen et al. [33] were able to raise the vitamin C content of maize leaves and kernels about 2-fold by increasing expression of the enzyme responsible for recycling ascorbate from one of the products (dehydroascorbate) of vitamin C oxidation.

4. Metabolic engineering approaches for mineral biosynthesis

More than 20 minerals are required as part of the human diet [89]. Iron, zinc and iodine are the mineral elements most frequently lacking in diets, but elements such as calcium, magnesium and selenium are also deficient in the diets of some populations. GM approaches to increasing the amounts of minerals in food crops have focused mostly on iron, zinc and selenium to date (Table 1). However, other modifications of food functionality, such as introduction of fructans (see below), may also affect the absorption of minerals from the diet.

Iron deficiency has been estimated to affect about 30% of the world population, making it the most widespread nutrient deficiency [41,80]. Key factors for supply of iron are not only its absolute amounts in food but also the presence or absence of compounds that affect its bioavailability, and these are a particular problem for rice-based diets. Although there is some variation in iron amounts in the available rice germplasm, issues with the bioavailability of iron may be hard to address by non-GM breeding [80]. Using GM, there has been little progress in engineering increased iron uptake into the plant as a whole, but there has been good progress in increasing the distribution of the iron into the edible parts and improving bioavailability factors. Combinations of current approaches might increase available amounts of iron in rice at least 2 to 3-fold, which could make a significant impact on iron deficiency for some populations [40,80]. However, metabolic engineering to raise iron (and zinc) content may only come fully into its own when there is

a better understanding of how the various system components, such as uptake, translocation and sequestration, contribute to plant mineral homeostasis.

The amount of selenium in the diet varies greatly with selenium concentration in soils. Increasing amounts of selenium in the diet is essential for some populations, while for others the amounts of selenium in soil contribute to selenium toxicity. Metabolic engineering approaches have been targeted at increasing selenium uptake into plants, and storage in non-toxic forms, both for phytoremediation and to supply adequate dietary selenium. Approaches that either release more selenium from amino acids or increase amounts of methylated selenium compounds have proven successful [43,44].

5. Metabolic engineering approaches for nutraceuticals/functional foods

To date, successful engineering for foods with enhanced amounts of nutraceuticals has been limited to a relatively small group of compounds, principally flavonoids, fatty acids and carotenoids, and for carotenoids this has mostly been focused on provitamin A production. One reason for the small group of nutraceutical compounds targeted is that many of the suggested beneficial compounds are produced by biosynthetic pathways for which there is limited information. A further important aspect, however, is the lack of medical information to guide metabolic engineering endeavours. The desired amounts of vitamins such as provitamin A may be well-known, but such information is lacking for many of the nutraceuticals that have attracted interest over the past few years – for example, on which specific compounds are the most beneficial, what glycosylation patterns are preferable, what are recommended amounts in food, how the background food matrix may impact on bioavailability or bioefficacy, and whether there are antinutritive impacts or toxic associations.

The initial focus on antioxidant properties of plant metabolites would have supported aiming for a general increase in the amounts of a broad range of phytochemicals with high antioxidant/anti-inflammatory potential. However, more details are now emerging on the particular interactions with the cellular machinery, especially gene regulation pathways [1]. The action of isoflavonoids as phytoestrogens has been known for some time, but there is now evidence for precise effects of other flavonoids. Anthocyanins, for example, enhance adipocytokine secretion and adipocyte-specific gene expression, and this may be an important action in reducing the risk of developing obesity and insulin resis-

tance [90,91]. Such research may help direct metabolic engineering projects for production of particular metabolites at recommended levels. However, these studies usually research only a single component of a complex physiological response, and also commonly use only a small selection of phytochemical structures. The anthocyanins illustrate the challenge of variation in the plant material, as there are now over 500 reported anthocyanin structures from plants [92].

Nutrigenomics offers the prospect of a greater understanding of the complex response of the genome to dietary components, through defining alterations in transcript, metabolite and proteome profiles following changes in nutrient or bioactive supply [1]. Although it will still be challenging to link individual components in a complex diet to a defined benefit for health, especially considering the added complication of an individual's genetic and environmental variation, in the longer term the ability of nutrigenomics to consider multiple variables may help in the development of novel functional foods. Some of the new plant lines being developed may assist with these studies. For example, conjugated linoleic acids (CLA) have a range of health benefits in animal studies, including reducing body fat gain and suppressing development of hypertension. Kohno-Murase et al. [50] recently generated transgenic rice lines that accumulate a single isomer of *trans*-10, *cis*-12 CLA in addition to the normal fatty acid profile, providing ideal material for model animal experiments to test increasing CLA intake within an otherwise unaltered diet.

Notwithstanding the current limitations on metabolic engineering of plant metabolite levels for benefits beyond nutritional contribution, there has been considerable success for the well-defined biosynthetic pathways for flavonoids and fatty acids. Some progress on GM of flavonoids was mentioned earlier, but the true extent of progress can be illustrated by examining the example of tomato. Many of the GM approaches proven in model systems such as *Arabidopsis* and *Petunia* (reviewed in [6]), have now been applied to this important food crop. Commonly, tomato fruit contain the pale yellow flavonoid naringenin chalcone, in addition to large quantities of the red carotenoid lycopene. Introduction of a transgene for the enzyme chalcone isomerase (CHI), which uses naringenin chalcone as a substrate, triggered the accumulation of flavonols in fruit [53]. By using a transgene for both CHI and the flavone synthase, flavone accumulation was enabled [54]. Overexpression of the enzyme that synthesises chlorogenic acid, hydroxycinnamoyl-CoA quinate:hydroxycinnamoyl transferase, resulted in accumulation of large amounts of chlorogenic acid with no

effects on the amounts of other soluble phenolics [51]. The introduction of a transgene for the MYB TF ANT1 produced transgenic plants with anthocyanin biosynthesis in the leaves and fruit [55], although anthocyanin production in fruit was only in small patches. It would be anticipated from the results with model systems that introduction of MYB and bHLH TF transgenes together would result in large amounts of anthocyanins in the fruit [6]. However, expression of transgenes for the maize C1 (MYB) and LC (bHLH) in tomato did not induce anthocyanin production in fruit, but rather increased flavonol amounts about 20-fold [93]. Fruit-specific RNAi down-regulation of the DET1 photomorphogenesis regulatory factor or overexpression of the CRYPTOCHROME2 photomorphogenesis factor resulted in transgenics with increased amounts of both flavonoids and carotenoids in the fruit [60,61]. In addition to manipulating amounts of these endogenous compounds, it has been possible to introduce novel phenolic biosynthetic activities into tomato. Specifically, the introduction of the enzymes stilbene synthase or polyketide reductase led to the accumulation of resveratrol (aglycone and glycosides) and 6'-deoxychalcones, respectively, in fruit of transgenics [54].

For fatty acids the focus has been on the production of long-chain polyunsaturated fatty acids (LC-PUFAs). The plant oils used for human consumption are formed of essentially the saturated palmitic (C16:0) and stearic (C18:0) acids and the unsaturated oleic (C18:1), linoleic (C18:2) and α -linolenic (C18:3) acids [94,95]. However, there is considerable evidence supporting the health-promoting effects of diets containing appropriate quantities of LC-PUFAs, such as arachidonic acid (ARA; 20:4), eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), which are only directly available from non-plant sources. There has been particular interest in the ω -3-LC-PUFAs that are abundant in fish oils, such as DHA and EPA, and the importance of EPA has been reviewed recently in detail by Sayanova and Napier [95]. Many of the genes involved in PUFA biosynthesis are now available from a range of organisms and these have enabled experiments to engineer oil-seed plants for the production of LC-PUFAs (Table 1). Examples of successful metabolic engineering of crop plants include the production of ARA and EPA in seeds of linseed (*Linum usitatissimum*) [49] and ARA, EPA, DHA and other non-host fatty acids in seeds of soybean (*Glycine max*) [47,48] and mustard (*Brassica juncea*) [46]. Only in some of the more recent studies, however, have substantive amounts of C20-PUFAs been formed, probably due to metabolic complexity arising from the introduction of these abnor-

mal compounds into the fatty acid biosynthesis and storage processes of higher plants [94]. The recent studies have obtained impressive amounts of LC-PUFAs in oil-seed species by using multiple transgenes. For example, Chen et al. [47] generated transgenic soybean that accumulated non-host PUFAs (including ARA) to 8.4% of total seed fatty acids by seed-specific expression of transgenes for $\Delta 5$ -desaturase, $\Delta 6$ -desaturase and a fatty acid elongase, and down-regulation of the host's $\Delta 15$ -desaturase. Kinney et al. [48] obtained up to 20% EPA of soybean seed oil by expression of the $\Delta 6$ -elongase and $\Delta 5$ -desaturase from the filamentous fungus *Mortierella alpina*, the $\Delta 15$ -desaturase from *Arabidopsis* and a $\Delta 6$ -desaturase and $\Delta 17$ -desaturase from the water mould *Saprolegnia diclina*. The subsequent addition of a $\Delta 5$ -elongase and $\Delta 4$ -desaturase was required for DHA production. Wu et al. [46] used a construct that expressed nine fatty acid biosynthetic genes to get large ARA and EPA amounts and some DHA production in mustard seeds. Overall, the experiments to date suggest extensive gene modification may be required to confer LC-PUFA biosynthesis into target species, and that variations in the endogenous fatty acid biosynthesis and storage pathways in different plant species may require development of species-specific protocols. Furthermore, in none of the experiments to date have large amounts of DHA production been achieved. Use of genes for LC-PUFA biosynthetic enzymes with additional activities may assist in increasing DHA amounts [96].

6. Metabolic engineering approaches for functional foods to improve gut health

The gastrointestinal tract is a key area for mediating the action of dietary plant material on human health. Environmental factors have a particularly large influence on a range of gut disorders, such as irritable bowel syndrome, and modifying dietary components could help to prevent or treat of such chronic conditions [1,97]. Foods and food products designed to benefit gut health may include addition of micro-organisms (probiotics), digestion-resistant carbohydrates (as prebiotics and dietary fibre) or specific bioactive compounds, such as phenolics. At present, the metabolic engineering of plants for non-nutrient based improvement of human gut health is at an early stage. However, there have already been notable achievements in modifying biosynthesis of carbohydrates that impact on gut health, specifically altering the type of starch accumulated and modification or introduction of fructan biosynthesis. Fructans are soluble carbohydrates comprised of fructose polymers, and occur as carbon storage molecules in plants of sev-

eral phylogenetically diverse families [98]. In some plant species they occur in large amounts in specialised storage organs, with important commercial examples including chicory (*Cichorium intybus*), Jerusalem artichoke (*Helianthus tuberosus*), leek (*Allium ampeloprasum* var. *porrum*) and onion (*Allium cepa*). Fructans cannot be digested directly by humans, but are fermented by the gut bacteria. There is evidence for a range of health benefits from inclusion of fructans in the diet [98]. It is thought that they have prebiotic qualities, acting as a food supply for, and encouraging the abundance of, beneficial gut bacteria such as *Lactobacilli* and *Bifidobacteria* species. There is also evidence, including from extensive animal trials, that fructans have beneficial effects on mineral absorption, blood lipid composition, and prevention of colon cancer, through prebiotic and independent mechanisms [98]. Extracted fructan, often obtained from chicory, is already in use as a functional food ingredient, as a soluble low-calorie fibre with associated human health benefits.

The potential to use high-fructan crops in the development of functional foods has encouraged GM approaches for the introduction of fructan biosynthesis into additional plant species, and over the past 10 years there has been much success for this aim. Notable examples for crop species include transgenic sugar beet (*Beta vulgaris*) and potato plants with significant fructan production in their tubers [70,71,74–76]. Furthermore, cDNAs for fructan biosynthesis enzymes have been used to modify the type of fructans that occur in current fructan-producing species, including introducing onion- or barley-type fructans into chicory [72,73]. This could have significant health-implications, as onion-type fructans may have a stronger prebiotic effect than the fructans found in commercially available chicory extracts [98]. Cairns [99] has reviewed the prospects for growing transgenic crops with metabolically engineered fructan production, including why the fructan amounts resulting may be lower than those of endogenous carbohydrates.

Starch is classified into two polymer types, amylose and amylopectin, based on the nature of the linkages between the individual glucose sub-units. In cooked foods, starch with a high-amylose content can be more resistant to digestion, and may have a range of benefits for gut health. GM potatoes have been produced that contain two antisense RNA constructs targeting the A and B isoenzymes of starch-branching enzyme II [77]. These lines produce tubers with very high-amylose content (up to 75% amylose), including under field conditions. Similarly, Regina et al. [78] produced wheat lines with an increased proportion of amylose-type starch (>70% amylose) by RNAi reduction of starch-branching

enzyme II activity. The impact of the new wheat phenotype on the large-bowel health of rats was then assessed using a dietary trial. The use of the high-amylose GM wheat in the diet improved a range of measures of gut health, including digesta-mass and short-chain fatty acid content, suggesting that this is a useful approach for increasing levels of digestion-resistant carbohydrate in food crops.

The role of phenolics, such as proanthocyanidins (condensed tannins), lignins and lignans, in gut health in humans is not clear, but there is evidence of beneficial effects [97]. The biosynthesis of lignans is only partially characterised, but some of the lignan biosynthetic proteins have been identified, and in theory it should be possible to markedly increase lignan amounts in GM plants [100]. Indeed, overexpressing genes for enzymes early in the flavonoid biosynthetic pathway in transgenic flax (*Linum usitatissimum*) significantly increased total phenolic antioxidant amounts in foliage and seed, and there was an accompanying slight increase in lignan amounts [52]. There is extensive molecular data on the biosynthetic pathways for proanthocyanidins and lignins and, although there are no published studies with regard to human health, it is worth noting that there has been substantial progress on GM of these biosynthetic pathways for improving performance of ruminant digestive systems. Proanthocyanidin biosynthesis has been introduced into additional plant species, and plants have been generated with more easily digested lignin [4]. The introduction of these characters into leading pasture species could significantly improve the efficiency and lower the environmental impact of pastoral agricultural systems.

7. Metabolic engineering approaches for reduction of antinutrients or allergens

Some crop plants may produce undesirable compounds such as antinutrients, allergens or toxins, and preventing the production of these in the edible parts is a target for metabolic engineering. Cassava (*Manihot esculenta*) is an interesting example, as it is one of the major food crops in Africa. Significant problems associated with cassava as a food staple are its low tuber protein content, post-harvest losses, and a high content of the cyanogenic glucosides linamarin and lotaustralin. When tissue is disrupted, the cyanogenic glucosides are converted to compounds that include the toxin hydrogen cyanide. Appropriate processing of the crop can reduce amounts of hydrogen cyanide in the food, but generally also results in loss of proteins, vitamins, and minerals. Although conventional breeding has generated

cultivars with reduced generation of hydrogen cyanide, no cultivars eliminate cyanogenic glucoside production. Jørgensen et al. [79] used RNAi to prevent production of the cytochrome P450 enzyme that makes the first committed step in the biosynthesis of linamarin and lotaustralin, and generated transgenic plants with elimination of cyanogenic glucosides in the leaves (<1% of non-transgenic amounts) and a 92% reduction of cyanogenic glucoside amounts in tubers.

Table 1 includes other examples of using GM for reduction of toxins or antinutrients, specifically; improving iron bioavailability through reducing phytic acid amounts in rice [42] and the reduction of sinapate ester content in seeds of oil-seed rape (*Brassica napus*) [67]. Metabolic engineering has also been used to generate lines of coffee (*Coffea arabica*) with reduced caffeine production [101]. Proanthocyanidins can be regarded as antinutrients, particularly against iron uptake [102], even though they may also have positive bioactive properties. There are several cases in which proanthocyanidin biosynthesis has been increased using GM, mostly in model species or forage crops [4,103], but no examples for their reduction in food crops to promote nutrient bioavailability.

8. Non-transgenic methods for metabolic engineering of crops

There are alternative approaches for developing new crop cultivars with improved human health characters that do not alter the spectrum of compounds normally associated with the crop. Accessions are available for many crops, such as carrots, potatoes, rice, sweet potato and maize, with high levels of human health-related metabolites [89,104]. However, a focus on yield and field performance during crop breeding has often meant that these characters were not selected for when developing modern cultivars [104]. Gene technology methods that enabled the transfer of such traits from existing germplasm into leading agronomic cultivars would likely raise fewer concerns over public acceptance of the modified crops, and fit more easily within traditional crop breeding programmes. One approach is to use gene sequences for marker-assisted breeding, and some of the genes encoding regulatory proteins may be particularly useful in this regard [9]. However, an alternative approach, which retains the benefits of GM technology, is to undertake metabolic engineering using only DNA sequences sourced from within the target crop's gene pool. Such transfer of DNA sequences only between plants from within the same sexual compatibility group has been termed 'intragenics' or 'cisgenics',

as opposed to traditional transgenics [105–107]. It is possible to replace all of the required sequences for function of the *Agrobacterium* T-DNA with DNA sequences derived from the target crop, to give a plant (P)-DNA fragment. These vectors may be designed using bioinformatic searches of publicly available DNA databases for the target crop to identify sequences that mimic the essential components of the T-DNA, such as the T-DNA borders. The selectable marker gene for identification of transformation events may also be of plant origin, for example the acetohydroxyacid synthase gene conferring tolerance to chlorsulfuron [106], or may be removed through a subsequent recombination process [105]. The effectiveness of such P-DNAs has already been demonstrated for crops, for down-regulation of polyphenol oxidase activity in intragenic potatoes [105]. Indeed, potato offers a good example of the possibilities for applying intragenics to crop plants. It is a crop widely grown throughout the developing and developed world, and is eaten by more than 1 billion people (<http://www.fao.org>). Although potatoes contain large amounts of vitamin C and significant amounts of thiamin, niacin and folate, their selenium and zinc content is generally less than optimal for human nutrition, and most commercial cultivars are low in carotenoids and flavonoids. However, landraces of potato are known with enhanced carotenoid or anthocyanin content [108,109] that could be sources of desired vitamin and nutraceutical characters, and it is likely that similar variation may be present for other key metabolites. Breeding of potatoes using traditional means is made more difficult by the complex genetic background of commercial lines, and intragenics may offer a route for more rapid development of new cultivars with improved human health characters. Besides the transfer of loci for improved traits from potato accessions into modern germplasm, gene inhibition-based experiments could also be accomplished with intragenic technology, such as the inhibition of ϵ -lycopene cyclase production to increase amounts of both total carotenoids and β -carotene [26].

9. Concluding comments

Now that there has been success with the engineering of some vitamin and nutraceutical pathways, the next logical step is the ‘stacking’ or ‘pyramiding’ of such transgenic traits. This is the target of a number of major programmes for some key crops. Details of programmes on banana, cassava, rice and sorghum can be found at the website for the “Grand Challenges in Global Health” initiative (<http://www.gcgh.org>). For rice, the aim is to combine the Golden Rice trait with gene

technology for increased vitamin E, iron and zinc, as well as an improved amino acid balance in grain protein content [84]. For a few species, such as potato, *Agrobacterium*-mediated gene transfer occurs with sufficient efficiency to allow development of GM plants without the need of a selectable marker, facilitating pyramiding of human health traits. However, for most species, systems are required to allow multiple transformation events, for example removal of the selectable marker gene in mature plants. Besides the challenge of obtaining stable expression of all the various transgenes required, there may be complications due to interacting biosynthetic pathways. For example, the pathways to carotenoids and tocochromanols share the precursor geranylgeranyl pyrophosphate.

The debate over acceptability and safety of GM crops is beyond the scope of this review, but crops with modified nutritional components are likely to be a focus both for proponents and opponents of GM. Advances in understanding of the triggers of disease, or even behaviour, and the possibility of linking these to nutrition through nutrigenomics, will add further complexity to the debate. A safety aspect often considered with regard to GM crops is the specificity of the new trait conferred. A concern for any breeding programme aimed at modifying activity of secondary metabolite pathways, whether using GM approaches or not, is the potential for altering production of non-target compounds. Metabolic pathways in plants often share a small number of common precursors, and altering production of an enzyme in one pathway may impact on substrate supply to other pathways. A simple example is that inhibiting production of the enzyme flavonol synthase can cause an increase in amounts of anthocyanins, as flavonols and anthocyanins share the common precursor dihydroflavonols [110]. A more complex example comes from the over-production of tryptophan decarboxylase (TDC) in potato [111]. The increased conversion of tryptophan in the TDC transgenics appears to alter the substrate feedback regulation that controls tryptophan biosynthesis, promoting activity of anthranilate synthase, which makes tryptophan, and reducing activity of chorismate mutase, which supplies the precursors of the phenylpropanoid pathway. Consequently, TDC transgenic potatoes had reduced phenylpropanoid production and increased susceptibility to the pathogen *Phytophthora infestans*. Given these considerations, metabolite and transcript profiling is a sensible step for GM plants with modified production of human health-related metabolites [112]. Such studies to date suggest that non-target changes may be uncommon or minor events. Indeed, in the case of potato lines mod-

ified for carbohydrate production or other characters, the metabolite profile differences between GM and non-GM lines were less than those found between different non-GM cultivars [113,114].

Targeted metabolic profiling could also be applied to identify varieties of crops, whether transgenic or non-transgenic, with the highest levels of desirable human health-related metabolites. Metabolic profiling has already been used for two pathways, flavonoids and carotenoids, to identify lines of transgenic or mutant tomato plants with high antioxidant content [115]. The extension of this, as we learn more about the interaction of bioactive compounds with human health through nutrigenomics and other approaches, is the use of metabolomics to screen large numbers of compound types in different cultivars for the development of personalised foods with medicinal benefits. Approaches that combine into one technique measuring amounts of the target compounds with assays of bioactivity may assist in automation of some of the process [116].

In summary, there has been impressive success in the metabolic engineering of crops for biofortification, but only for a small group of target compounds to date. Knowledge of the molecular genetics of plant biosynthetic pathways is increasing rapidly, and many new DNA sequences have been published in the past few years for enzymes involved in the biosynthesis of human health-related compounds. For example, genes involved in the biosynthesis of vitamins B₂ and B₅ have now been identified from arabidopsis [117,118]. This rapid progress is not just in model research species, such as arabidopsis and rice, but in a wide range of crops. Recent examples for bioactives include the identification of sequences for capsaicin synthase, which produces the pungency factor capsaicin in hot peppers [119], curcuminoid synthase for the biosynthesis of curcumin in turmeric (*Curcuma longa*), and hydroxycinnamoyl-CoA thioesterases involved in the biosynthesis of gingerols in ginger (*Zingiber officinale*) [120]. Given the progress in identifying the biosynthetic genes, wide-ranging success with metabolic engineering of human health-related compound biosynthesis in plants would be expected in the near future.

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