Chapter 10

Genetic Engineering to Enhance Crop-Based Phytonutrients (Nutraceuticals) to Alleviate Diet-Related Diseases

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Abstract

Nutrition studies have provided unambiguous evidence that a number of human health maladies including chronic coronary artery, hypertension, diabetes, osteoporosis, cancer and age- and lifestyle-related diseases are associated with the diet. Several favorable and a few deleterious natural dietary ingredients have been identified that predispose human populations to various genetic and epigenetic based disorders. Media dissemination of this information has greatly raised public awareness of the beneficial effects due to increased consumption of fruit, vegetables and whole grain cereals—foods rich in phytonutrients, protein and fiber. However, the presence of intrinsically low levels of the beneficial phytonutrients in the available genotypes of crop plants is not always at par with the recommended daily allowance (RDA) for different phytonutrients (nutraceuticals). Molecular engineering of crop plants has offered a number of tools to markedly enhance intracellular concentrations of some of the beneficial nutrients, levels that, in some cases, are closer to the RDA threshold. This review brings together literature on various strategies utilized for bioengineering both major and minor crops to increase the levels of desirable phytonutrients while also decreasing the concentrations of deleterious metabolites. Some of these include increases in: protein level in potato; lysine in corn and rice; methionine in alfalfa; carotenoids (β-carotene, phytoene, lycopene, zeaxanthin and lutein) in rice, potato, canola, tomato; choline in tomato; folates in rice, corn, tomato and lettuce; vitamin C in corn and lettuce; polyphenolics such as flavonol, isoflavone, resveratrol, chlorogenic acid and other flavonoids in tomato; anthocyanin levels in tomato and potato; α-tocopherol in soybean, oil seed, lettuce and potato; iron and zinc in transgenic rice. Also, molecular engineering has succeeded in considerably reducing the levels of the offending protein glutelin in rice, offering proof of concept and a new beginning for the development of super-low glutelin cereals for celiac disease patients.

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Introduction

Nutrition and human health have become synonymous due to the realization that certain nutrients when consumed in specific quantities may contribute to optimal health.[1] Poor nutrition is particularly relevant in regard to the diet-related diseases.[2] Targeting specific dietary treatment as therapeutic has important clinical and economic relevance, particularly in high prevalent diseases including coronary artery disease, hypertension, diabetes, osteoporosis and possibly cancer in adults and celiac disease (CD), cow's milk protein allergy (CMPA), galactosemia and glycogen storage disorders in children.[3] Prevention and therapy of age- and lifestyle-related diseases by individualized tailoring of optimal diets or nutrients seem conceivable but will need scientific validation through human nutrition trials and evaluation of dosage with minimal side effects.

Majority (97%) of the genes associated with human disease have been classified under monogenic diseases because each is a result of a dysfunctional gene.[4] Although human health disorders are basically genetic, there is a definite interplay of disorders/disease with either insults contributed by consumption of certain, commonly used foods, or preventive strategies involving dietary intervention, through nutritionally-enhanced food. In several cases, the offending as well as favorable dietary agents over and above genetic predisposition have been identified in human populations.[5-7] For instance: a gluten-free diet reverses the adverse health effects in CD; restricting intake of galactose and lactose helps in the control of galactosemia; and milk-free diet remedies CMPA. Diseases classified as polygenic in nature such as epithelial cancers, diabetes and heart disease seem to be ameliorated by the intake of dietary antioxidants, vitamins and other phytonutrients.[8,9] Dietary phytonutrients constitute variable antioxidants that prevent accumulation of damaging oxidants (reactive oxygen species, ROS) potentially preventing oxidation of cellular and sub-cellular macromolecules. Thus, phytonutrients, particularly those with antioxidant properties, have become a household name and are in demand as desirable nutraceuticals worldwide (see Chapter by Adams and Adams).

Analysis of about 200 dietary studies concluded that fruit and vegetable consumption is significantly protective against various types of cancers.[10] Notably, persons who consumed lower quantities of fruits and vegetables were at twice the risk of experiencing cancer than those that consumed higher proportion.[10] Nonetheless, a wide variation in response to food components as modifiers of cancer risk and tumor behavior has also been observed in other preclinical and clinical studies. These inconsistencies can be due to a number of factors, including: (i) the kind and level (based on the serving size) of antioxidant constituents of food; (ii) specificity and temporal relationships among dietary constituents as modifiers of molecular events; (iii) multi-factorial nature of signals that determine phenotypic expression; (iv) the nature of antioxidant response elements (AREs) present in the human genome which are polymorphic.[11] Clearly, there is a need for a better understanding of the relationship between dietary intervention and disease progression to develop fundamental understanding of disease-related process(es) impacted by one or more dietary constituent.[12]

There is a major gap in our understanding of the exact pathophysiology and a paucity of scientific tools to target prevention, enzyme replacement for specific deficiency, or gene therapy. However, definite progress has been made where it is possible to effectively manage several human disorders by dietary therapy and potentially prevent other serious diseases by consumption of nutritionally-enriched vegetables and fruits. The concept of developing nutritionally functional food requires: (1) the understanding of the mechanisms of prevention and protection; (2) the identification of the biologically active molecules and (3) the demonstrated efficacy of these molecules with human subjects. Accurate information is also highly desired to have in place about the required daily allowance (RDA) for effective phytonutrients, to name a few, vitamin C, carotenoids such as lycopene, β-carotene and lutein, phenolics, flavonoids, minerals, etc., in order to design biotechnological strategies for manipulating their contents in vegetables and fruits. Nutritional therapies are prone to several limitations currently existing either in achieving an appropriate control—prevention of ongoing organ damage, best survival and normalcy—or in acceptability, convenience and affordability.

Databases are growing with information on the content of phytonutrients in edible vegetables and fruits as well as with transgenic technology developments for enhancing the nutritional content of vegetable crops via engineering of specific metabolic pathways. Metabolic pathway engineering approaches have demonstrated the power of genetic manipulation in enhancing the content of nutrients beneficial for human health in transgenic crops. In the same vein, suppression of key genes to inhibit production of allergenic proteins or toxins in crops is highly sought.

Diet and Human Diseases

Diseases such as CD, galactosemia and phenylketonuria occur because of intolerance in a certain population to specific food components (Table 1).[2] Preventive measures include dietary restrictions and eliminating foods containing the disagreeable compound. CD is caused in genetically susceptible individuals (HLA predisposing genotypes, DQ2 and DQ8) who are sensitive to gluten, the storage protein present in wheat, barley and rye. CD is a common lifelong disorder in European-origin countries, affecting approximately 1% of the general population and prevalent also in North Africa, Middle East, India and Saharawi people.[6] Continued consumption of gluten-rich diets in many developing countries will result in increasing the frequency of this disease in the future. CD manifests as chronic diarrhea, anemia, growth failure, vitamin (fat and water soluble) deficiencies and other systemic disorders.[7] If left untreated, higher incidence of malignancy is seen. A definitive treatment for CD is life-long elimination of gluten from the diet. Since wheat, barley or rye form part of common diets, the administration of gluten-free diet is expensive and difficult, inconvenient, socially problematic and with high chances of noncompliance.[13] There is, thus, a need for better, convenient and long lasting treatment options.

Galactosemia is an autosomal recessive disorder of galactose metabolism, caused by a deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT; EC 2.7.7.12).[14] Galactose accumulation leads to toxic metabolites that cause damage to patient's liver and central nervous system. It is in the neonatal period that its clinical manifestation occurs, via ingesting breast milk and top milk causing release and accumulation of galactose from lactose by the intestinal lactase. The afflicted children suffer from jaundice, hepatosplenomegaly, liver dysfunction, hypoglycemia, renal tubular dysfunction, muscle hypotonia, cataract and *Escherichia coli* sepsis (Tables 1 and 2). If left untreated, galactosemic patients rapidly develop liver cirrhosis and early death. GALT activity in red blood cells together with determination of urinary sugars and sugar alcohols (showing elevated concentrations of galactose and

Table 1. Diet-related monogenic diseases in humans

Disease	Cause	Therapy
Celiac disease (CD)	In genetically susceptible individuals upon eating gluten present in barley, rye and wheat	Gluten-free diet
Cow's milk protein allergy (CMPA)	Allergic disorder to milk protein, β-lactoglobulin	Milk-free diet supplemented with calcium, riboflavin and vitamin D
Galactosemia	Deficiency of galactose-1-phosphate uridyl transferase	Galactose and lactose-free diet
Glycogen storage disorder (GSD)	Dysfunction of enzymes in glycogen metabolism	Nasogastric feeding or augmented diet with corn starch
Phenylketonuria	Defective phenylalanine metabolism, defective or deficient phenylalanine hydroxylase	Phenylalanine-free diets supplanted with tyrosine

Modified from reference 2.

Table 2. Major types of glycogen storage disorders showing defect and organ involved

Type	Defect	Predominantly Affected Organ
I: *Types*: Ia,Ib,Ic,Id	Glucose-6-phosphatase system deficiency	Liver
II: (4 onset forms) Pompe disease	Defective lysosomal α-glucosidase	Muscle ± cardiac muscle
III: (6 forms)	Amylo-1,6-glucosidase deficiency	Only liver Type IIIa: Both liver + muscle
IV: (Many forms) Andersen disease/Amylopectinosis	Deficiency of the glycogen-branching enzyme	Cirrhosis of liver: Both liver + muscle
V: (2 forms) McArdle disease	Muscle phosphorylase deficiency	Muscle
VI	Liver phosphorylase deficiency	Liver
VII: (2 subtypes)	Phosphofructokinase deficiency	Muscle
IX	Tissue-specific phosphorylase kinase deficiency	Liver
0: (2 subtypes)	Glycogen synthase deficiency	Liver
XI	Defect in glucose transporter 2 (GLUT-2)	Liver

galactitol) are diagnostic tools to identify classical galactosemia patients. The only therapy for such patients is that galactose must be removed from the diet as soon as the diagnosis is made and patients put on lactose-free diet. Even when the diet restriction is followed, complications such as retarded mental development, verbal dyspraxia, motor abnormalities and hypergonadotrophic hypogonadism develop in the long term. In the current recommendations, the restriction on galactose-containing fruit and vegetables has not been specified.

CMPA is a common disorder due to the milk protein, β-lactoglobulin. This disorder makes infants suffer from gastrointestinal symptoms with or without skin and respiratory symptoms.[5,15] Disease symptoms are chronic diarrhea, vomiting, anemia, failure to thrive and sometimes bleeding per rectum.[15] A prevalence of 13% among children under 2 years of age with malabsorption has been reported from developing countries.[16] CMPA patients are put on milk-free diet under the supervision of a dietician, who provides advice on milk-free recipes together with a list of alternate products for adequate calcium, riboflavin and vitamin D intake to avoid development of deficiency. Thus, restriction of milk to mothers breastfeeding such children becomes at times necessary, which is cumbersome. CMPA children are fed soy formulas, extensive hydrolysates (casein, whey or mixed) and amino acid based formulas.[15,17] There is a need for alternative and better dietary therapy. Advances in food biotechnology can pave a way to develop β-lactoglobulin-free bovine/cow's milk or devise biological processing to eliminate allergenic protein fraction of milk.

Dysfunctional enzymes involved in the synthesis and/or degradation of glycogen lead to glycogen storage disorder (GSD), a disease characterized by abnormal inherited glycogen metabolism in the liver, muscle and brain. Based on each defective metabolic step in carbohydrate pathways gives rise to different types (I-XI) of GSD[18,19] (Tables 1 and 3). Detailed dietary management for various types of GSD disorder is summarized in Table 3. There is a need for genetically modified food item to suit the requirement of the GSD children and development of cornstarch that has a longer life at ambient temperatures.

Phenylketonuria is another monogenic diet-related disease, a genetic disorder caused by the deficiency of or defective phenylalanine-metabolizing enzyme, phenylalanine hydroxylase.[20,21]

Table 3. Dietary management and its importance in various types of glycogen storage disorders

Type	Management	Targeted Dietary Importance
I	Restriction of dietary fructose and galactose Challenge to maintain normal blood glucose levels: • Infusions—intravenous/nasogastric and • corn starch	++
II	Enzyme replacement therapy	–
III	Hypoglycemia: corn starch supplement	+
IV	Treatment of liver disease	–
V	Supportive	High protein diet
VI	Hypoglycemia: high carbohydrate diet + frequent feeding	+
VII	Supportive	High protein diet
IX	Hypoglycemia: high carbohydrate diet + frequent feeding	+
0	Night time corn starch to maintain normal blood glucose levels	+
XI	Restrict galactose and fructose intake. Corn starch for improved growth	+

Aromatic amino acids including phenylalanine are generated from phosphoenolpyruvate, which offers a branch point for the synthesis of pyruvate and oxaloacetate and that of the shikimate pathway.[22] Phenylalanine accumulation in the blood drastically increases the risk of neurological damage. Therefore, higher dietary levels of phenylalanine are not desirable for patients suffering from phenylketonuria.[2,23] Treatment of this disorder calls for diets absent in phenylalanine and supplemented with tyrosine.[21]

In addition to the above described, diet-induced monogenic diseases, there is more prevalence of polygenic diseases such as cancer, diabetes and heart disease, which are often linked to dysfunctional biological networks.[24] These diseases are not easily treatable. However, dietary intervention has a good potential in preventing their onset. A case in point is the recent demonstration of a positive effect of antioxidant supplementation in relieving pain and reducing oxidative distress in patients with chronic pancreatic disease, pancreatitis.[25] The prevention strategy will be furthered by the knowledge developed about the elucidation of how a single nutrient (nutraceutical) functions in a biological system and the nature of interactions between a mixture of nutrients in a diet and prevention of a disease.[1,26]

Phytonutrients and Antiproliferative Activity

Cell proliferation is a characteristic manifestation of any malignancy and, therefore, anti-proliferative activity of individual antioxidants and diets is a means to discover potential anti-cancer agents. Because phytonutrients in fruit and vegetables seem to have synergistic effects on the antioxidant activities and may reduce the risk of chronic diseases, there is considerable interest to test their performance in mitigating cell proliferation.[27,28] Understandably, research has also intensified on analyzing and enhancing nutritional, antioxidant metabolites in food crops. It is well known that vegetables, fruits, nuts and various herbs are dietary sources of one or more of the following nutraceuticals: vitamins C, B and E, β-carotene, lycopene, polyphenols, flavonoids, folates, isothyocyanates, glucosinolates and minerals. Nonetheless, antioxidant capacity of fruits is a function of genotype/cultivar, growth condition:[29-32] the content of lycopene, ascorbic acid (vitamin

C) and phenolics in different tomato genotypes is variable[33] while the phenolic (flavonoid and capsaicinoid) content of hot pepper is affected by the color and maturation stage, the antioxidant activity at red stage being greater than the green maturity stage.[34] Likewise, antioxidant capacity due to stilbenes (resveratrol, pterostilbene and piceatannol) in various selections and cultivars of berries from USA and Canada indicated highest resveratrol content in grapes and berries.[35] These findings highlight the importance of taking into consideration the fact that antioxidant capacity of fruits significantly varies between genotypes, species and developmental stage, which can affect their nutritive value. Also of relevance is the finding that pomegranate juice shows higher bioactivity than individual polyphenols extracted from the fruit.[36]

Resveratrol was found to have growth inhibitory and antiproliferative activity in vitro on human promyelocytic leukemia (HL-60) cells.[37] Among fruit extracts tested on in vitro proliferation of HepG2 human liver-cancer cells, cranberry had highest inhibitory activity followed by lemon, apple, strawberry, red grape, banana, grapefruit and peach.[38] Also, it has been noted that the antiproliferative effects of fruit extracts and their antioxidant capacity are not always tightly correlated. Total antioxidant capacity (due to total phenolics and flavonoids) in fruit extracts of eight different strawberry cultivars did not correlate with their antiproliferative activity.[39] Similar results were previously obtained with raspberries.[40] These studies point out that multiple and synergistic interactions among chemicals promote antiproliferative activity of a fruit compared to an isolated antioxidant.[36, 41, 42]

Lycopene-rich foods lower biomarkers of oxidative stress and carcinogenesis in healthy and type II diabetic patients and prostate cancer patients, respectively. Dietary fats are recommended together with lycopene to enhance the beneficial aspects of lycopene.[9] Rats fed 0.7 mg/d lycopene for 4 days showed a significant reduction in the androgen status of the animal.[43] Induced oxidative stress in COS-7 cells was antagonized by blue berry extracts that lowered activation of cyclic AMP responsive element binding protein (pCREB) and possibly pPKCgamma kinase.[44]

Polyphenols[45,46] and dietary flavonols[47] were found to bind human lower density lipoproteins (LDL) whose cellular oxidation is considered a key step in atherogenicity.[48] Intake of grape juice,[49,50]tea, or red wine,[51] or an antioxidant nutrient like vitamin E (tocopherol)[52] enriches LDL levels and protects them against oxidation.

Genetic Engineering to Improve Nutrient (Nutraceutical) Content in Produce

Staple foods such as rice, corn, wheat and cassava are relatively poor sources of health-promoting nutrients. In the developing world this contributes to health problems because the traditional diets are deficient in one or more essential nutrients, causing malnutrition and disease. Thus, a simple solution entails food biofortification with externally supplied vitamins and antioxidants, or via genetic manipulation of nutraceuticals in staple food. Off-the-shelf supplements are commonly consumed because it is perceived that they improve whole body health. However, the bioavailability of nutrients in a diet determines how much of a good nutrient's potential is realized.[53] The refined tools of genomics, proteomics and metabolomics are now in vogue to enable in-depth studies on the relationships between diet, genetics and metabolism. A biotechnological approach is to genetically engineer the required threshold of nutrients in the edible portions of a grain crop or a fruit/vegetable crop. Thus, development of 'golden' rice with added pro-vitamin A, β-carotene and protein is an innovation that might help conquer malnutrition in the developing world.[54,55] A recent advance has been the development of transgenic multivitamin corn, a study that introduced four cDNAs encoding enzymes in the biosynthetic pathways of vitamins β-carotene, ascorbate and folate.[56] The transgenic corn endosperm accumulated up to 59.32 μg/gDW of β-carotene, 22.78 μg/gDW of lycopene, 106.94 μg/gDW of vitamin C (ascorbate) and 35.76 μg/gDW of zeaxanthin. We summarize below examples of the application of genetic engineering and biotechnology approaches in enhancing the levels of various phytonutrients (nutraceuticals) in cereal, vegetable and fruit crops. Table 4 lists cereal crops and Table 5 lists vegetable and fruit crop—however, not all those listed have been elaborated upon. The results clearly indicate the power of genetic engineering in achieving the desired levels of nutraceuticals in crop plants.

Table 4. Genetically modified enhancement in nutrients of major crops

Target Nutrient/Crop	Gene Introduced/ Modified	Promoter Used	Transgenic vs. WT	Reference
Amino Acids/ Rice	*GluB* (RNA silencing)	CaMV35S (constitutive)	Low glutelin content than Lgc-1 mutant	57
	CTA-(altered) $tRNA^{lys}$	Ubi	41.42% vs. 0.81% lysine content of prolamines	58
Amino Acids/ Corn	*LKR/SDH*/Corn (RNAi suppression)	B32 (endosperm specific)	Free lysine: ~650 ppm vs. 30 ppm	59
	SB401/Potato	P19Z (seed specific)	Lysine: 54.8% vs. 16.1%; total protein content: 39% vs. 11.6%	117
	Synthetic porcine α-lactalbumin	γ-zein (endosperm specific)	Lysine: 47% vs. 29%	118
	LKR1/SDH/Corn (dsRNA) + *CordapA/ Corynbacterium glutamicum*	Pe35S	Lysine: 4000 ppm vs. 100 ppm	60
Carotenoid/ Rice	PSY/daffodil	CaMV35S or Gt1 (endosperm specific)	Phytoene: 0.74 μg g^{-1}DW (with pGt1); 0.32 μg g^{-1}DW (with CaMV35S); nd in WT	63
	Psy/daffodil + *Crt1/ Erwinia uredevora*	Gt1 and CaMV35S	Higher β-carotene and lutein and zeaxanthin	53
	Psy/daffodil + *Crt1/E. uredevora* (construct 1); *Lcy*/daffodil (construct 2)	Gt1 and CaMV35S (construct 1); Gt1 (construct 2)	From variable carotenoid to only β-carotene accumulation: 1.6 μg · g^{-1} in heterozygous lines	53
	psy/corn + crtI/daffodil	Glu1 (endosperm specific)	Total carotenoids: 37 μg g^{-1}DW; β-carotene: 84 μg · g^{-1} DW (nd in WT)	54
Carotenoid/ Corn	*Psy1*/corn + *crt1/E. uredevora*	LMW glutelin/ wheat and D-hordein/barley (endosperm specific)	β-carotene: 4.79 vs. 0.09 μg g^{-1}DW; Lycopene: 22.78 vs. 0 μg g^{-1}DW	55
	crtB + *crt1/E. uredevora*	γ-zein	Provitamin A: 7 vs. 0.39 μg g^{-1}DW; total carotenoids: 33 vs. 1 μg g^{-1}DW	66

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Table 4. Continued

Target Nutrient/ Crop	Gene Introduced/ Modified	Promoter Used	Transgenic vs. WT	Reference
Folate/Rice	*GTPCHI* and *ACDS*	A single T-DNA expression cassette	Folate: 38.3 vs. 0.38 nmol g^{-1}FW; 5methyl-tetrahydrofolate, 89% of total folate	89
	GTPCHI/Arabidopsis	glb-1 (endosperm specific)	Pterin: 0.23 vs. 0.92 nmol g^{-1}FW	89
Folate/Corn	*GCH1*/*E. coli*	D-hordein	1.94 vs. 0.94 μg g^{-1}DW	55
Ascorbate/Corn	*DHAR*/wheat	Ub-(ubiquitin) or Sh2 (shrunken 2)	Ascorbate: ~80 vs. 42 nmol g^{-1} FW; 20-106 fold increase in DHAR activity	93
	DHAR/rice	D-hordein	106 vs. 17.53 μg g^{-1}DW	55

nd: not detected; DW: dry weight; FW: fresh weight.

Proteins and Amino Acids

Major Crops

The seed storage protein, glutelin, which is a cause for CD, commonly occurs in cereals. Its reduction is therefore a promising avenue for CD patients. In this context, Low Glutelin Content-1 (*Lgc1*) is a dominant mutation that reduces glutelin content in rice grain while increasing prolamine and other seed storage proteins.[57] RNA interference (RNAi) approach was used to silence glutelin *GluB* gene, producing low glutelin protein phenotypes of rice (Table 4).[58] Among the transformants characterized, *GluB4*-Δ*GluB5* and Δ*GluB5* were found to accumulate glutelin content lesser than the LGC-1 mutant. These authors suggested that these transgenic lines are a good resource to produce "super low-protein rice" and may be more beneficial than LGC-1 for kidney disease patients.

The content of essential amino acids such as lysine is low in staple crops such as rice. To increase the lysine/amino acid content of rice seeds, $tRNA^{lys}$ with altered anti-codons (chimeric $tRNA^{lys}$) was expressed under ubiquitin promoter in rice callus.[59] Such a strategy resulted in significant increase in the content of lysine without affecting the quantity or the quality of the storage proteins. A different approach to increase lysine content was to inhibit its catabolism. Thus, an inverted repeat (IR) sequence corresponding to part of the saccharophine dehydrogenase (SDH) region of corn (*IR-SDH*) was introduced to enable endosperm-specific suppression of the corn bifunctional lysine degradation enzyme, lysine-ketoglutarate reductase and SDH (LKR-SDH), in transgenic corn kernels by RNAi.[60] This approach led to a 20-fold increase in free lysine content in homozygous transgenic corn kernels over the null control. These studies were complemented by a study that employed a single transgene cassette for simultaneous expression of the deregulated lysine degradation enzyme, *CordapA* and suppression of LKR/SDH, in transgenic corn.[61] This approach of combining LKR/SDH silencing and *CordapA* expression led to over 40-fold (4000 ppm in transgenic versus 100 ppm in nontransgenic control) accumulation of free lysine in corn grain.

Minor Crops

Potato tops the list of vegetable crops consumed in the world. Apart from being a rich source of starch and vitamin C, it lacks protein and other nutrients. In an effort to produce a high quality protein-rich potato, Amaranthus seed albumin gene, *AmA1*, was engineered and expressed

Table 5. Genetically modified enhancement in nutrients of vegetables and fruits

Target Nutrient/Crop	Gene Introduced/Modified	Promoter Used	Transgenic vs. WT	Reference
Amino Acids/Potato	*AmA1/A. hypochondriacus*	CaMV35S (constitutive) and GBSS (tuber specific)	Total protein: ~16.5 vs. 11.5 mg g^{-1}FW; 2.5-4 fold increase in essential amino acids: lysine, methionine, cysteine and tyrosine	61
Amino Acids/Alfalfa	*AtCGS*/Arabidopsis	Rubisco SSU	Methionine: 4846 vs. 2198 nmol g^{-1}FW; cysteine: 1873 vs. 782 nmol g^{-1}FW	62
Amino Acids-Choline/Tomato	*ySAMDC, Spe2*/Yeast	E8 (fruit specific)	Increase in glutamate, asparagine and choline	22
Carotenoid/Brassica	ε-*CYC/B. napus* (RNAi)	P35S (constitutive)	β-carotene: 90.76 vs. 0.49 μg g^{-1}FW; lutein: 76.22 vs. 3.30 μg g^{-1}FW	119
Carotenoid/Flaxseed	*crtB/P. ananatis*	CaMV35S and FAE1 (seed specific)	156.3 vs. 8.4 μg g^{-1}DW	71
Carotenoid/Canola	*crtB*/bacteria	Napin (seed specific)	1000 vs. 33 μg g^{-1}FW	69
β-Carotene/Canola	*crtI* + *crtb*/bacteria	Seed specific	857 vs. 5 μg g^{-1}FW	70
	DET1 (RNAi suppression)	CaMV35S	7 vs. 0.5 μg g^{-1}FW	120
Lycopene/Canola	*crtI* + *crtb/bacteria*	Seed specific	44 vs. 33 μg g^{-1}FW	72
β-Carotene/Tomato	*DET1*/Tomato (RNAi)	P119 (fruit specific)	20.5 vs. 2.4 μg g^{-1}FW	79
	β*Lcy/Arabidopsis*	Pds (fruit specific)	50.8 vs. 8.0 μg g^{-1}FW	73
	β*Lcy/Arabidopsis* + β*Lcy*/Pepper	Pds	63 vs. 5 μg g^{-1}FW	74
	Cry-2/Tomato	CaMV35S	101 vs. 78 μg g^{-1}DW	121
	CrtB/E. uredovora	PG (fruit specific)	1700 vs. 33 μg g^{-1}DW	77

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Table 5. Continued

Target Nutrient/Crop	Gene Introduced/Modified	Promoter Used	Transgenic vs. WT	Reference
	β-*cyclase/E. herbicola*	Atpl (chloroplast targeted)	286 vs. 69 μg g^{-1}DW	122
	Crt1/E. uredovora	CaMV35S	520 vs. 271 μg g^{-1}DW	76
Lutein/Tomato	*Cry-2*/Tomato	CaMV35S	36 vs. 23 μg g^{-1}DW	122
	β*Lcy*/Arabidopsis (antisense)	Pds	97 vs. 54 μg g^{-1}FW	73
	Cry-2/Tomato (gene silencing)	CaMV35S	1,353 vs. 775 μg g^{-1}DW	122
Lutein/Tomato	*DET1*/Tomato	2A11, P119, TFM7 (fruit specific)	2-fold	79
	CUL4/Tomato (RNAi) *HP1*/ Tomato (RNAi)	CaMV35S TFM7	300 vs. 150 μg g^{-1}FW	82
Lycopene/Tomato	*ySAMDC, Spe2*/Yeast	E8	2 to 3-fold: 150-175 vs. 25-50 μg g^{-1}FW	83
β-Cryptoxanthin, zeaxanthin/ Tomato	β*Lcy*/Arabidopsis + β*Chy*/ Pepper	Pds	24 μg g^{-1}FW (nd in WT)	74
β-Cryptoxanthin, zeaxanthin/ Tomato	β*Lcy*/Arabidopsis + β*Chy*/ pepper	Pds	24 μg g^{-1}FW	74
Carotenoids/Potato	*Or*/cauliflower	GBSS (tuber specific)	31 vs. 5.5 μg gDW^{-1}	85,86
	CrtB/E. uredovora	Patatin (tuber specific)	35 vs. 5.6 μg g^{-1}FW	72
	CrtI,CrtB and *CrtY*	Pat1 (tuber specific)	Total carotenoids: 114 vs. 5.8 μg g^{-1}DW; β-carotene: 47 vs. 0.013 μg g^{-1}DW;	123
	LCY-e/potato (antisense)	Patatin B33 (tuber specific)	Total carotenoids: 12272 vs. 4672 ng g^{-1}DW; β-carotene: 43 vs. 3 ng g^{-1}DW; zeaxanthin: 990 vs. 262 ng g^{-1}DW	124

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Table 5. Continued

Target Nutrient/Crop	Gene Introduced/Modified	Promoter Used	Transgenic vs. WT	Reference
Phytoene/Tomato	*crtB/E. uredovora*	PG	47 vs. 28 μg g^{-1}DW	77
	Psy-1/Tomato	CaMV35S	240.2 vs. 102 μg g^{-1}FW	125
Phytoene/Potato	*dxs/E. coli*	Patatin (tuber specific)	3.0 vs. 0.4 μg g^{-1}FW	126
Ketocarotenoid/Potato	*crtO/Cyanobacterium* (cotransformation of *zep* transformants)	CaMV35S	Ketocarotenoids accumulate up to 10-12% of total carotenoids	127
Ketocarotenoid/Carrot	*CrtO/H. pluvialis*	Ubi/CaMV35S/RolD	70% of total carotenoid converted to ketocarotenoids	87
Folate/Tomato	*GCH1*/mammalian	E8	Total folate: 4.5 vs. 2.3 nmol g^{-1}FW	90
	GCH1/Mammalian + *ADCS*/Arabidopsis	E8	25 vs. 1 nmol g^{-1}FW	91
Folate/Lettuce	*Gch1*/avian	CaMV35S	1.885 vs. 0.34 μg g^{-1}FW	92
Iron/Lettuce	*pfe*/Soybean	CaMV35S	398 vs. 227 μg g^{-1}DW	128
Ascorbate/Lettuce	Gul oxidase/rat	CaMV35S	4 to 7-fold	94
Tocopherols/Lettuce	γ *TMT*/Arabidopsis	CaMV35S	Over 2-fold in vitamin E; complete conversion of γ-tocopherol to α-tocopherol	107
Tocopherols/Soybean	*AtVTE3* + *AtVTE4*/Arabidopsis	Napin (seed specific)	α-tocopherol: 91.4 vs. 10.5%	106
	γ *TMT*/Arabidopsis	CaMV35S	4 fold increase in α-tocopherol	108
	γ *TMT/P. frutescens*	Vicilin (seed specific)	α-tocopherol: 450.1 vs. 43.9 pmol mg^{-1}FW; β-tocopherol: 55.7 vs. 4.3 pmol mg^{-1}FW; 4.8-fold higher vitamin E activity	110

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Table 5. Continued

Target Nutrient/Crop	Gene Introduced/Modified	Promoter Used	Transgenic vs. WT	Reference
Tocopherols/Oilseed	γ *TMT*/Arabidopsis	CaMV35S	α-tocopherol: 62 vs. 9.8%; γ-tocopherol: 35.84 vs. 86.82%	109
Tocopherols/Tomato	*hmgr-1*/Arabidopsis	CaMV35S	Phytosterol: 1800 vs. 1182 μg g^{-1}DW; campesterol: 161.7 vs. 96 μg g^{-1}DW; cycloartenol: 234 vs. 162 μg g^{-1}DW	78
Flavonoids/Tomato	*HQT*/Tomato	CaMV35S	Chlorogenic acid: 1.85 μg g^{-1}FW	96
	DET1/Tomato (RNAi suppression)	TFM7, 2A11 and P119	1.9 to 3.5-fold	79
	Cry-2/Tomato	CaMV35S	Total: 5.31 vs. 1.85 μg mg^{-1}DW	121
	chi/Petunia	CaMV35S	Quercetin 16.52 vs. 0.25 mg g^{-1}DW; kaempferol: 2.05 vs. 0.03 mg g^{-1}DW	95
Flavonoids/Potato	*DFR/P. hybrida*	CaMV35S	Chlorogenic acid: 2.5 vs.1.5 mg g^{-1}DW; pelargonidin: 0.4 vs.0.1 mg g^{-1}DW; petunidin: 3.0 vs. 0.5 mg g^{-1}DW	103
Flavonol/Tomato	*Chi*/Petunia	CaMV35S	1.9 vs. 0.09 mg g^{-1}DW	95
Flavonol/Tomato	*MYB12*/Arabidopsis	CaMV35S	Quercetin: 20 vs. 0.30 mg g^{-1} DW; kaempferol: 28 vs. 0.05 mg g^{-1} DW; chlorogenic acid: 1.17 vs. 0.04 mg g^{-1} DW	129
Anthocyanin/Tomato	*Del*/Snapdragon + *Ros1*/Snapdragon	E8	2.83 mg g^{-1}FW (nd in WT)	104
Isoflavone/Tomato	*IFS*/Soybean	CaMV35S	Genistin: 0.45 $nmol^{-1}$gFW; quercetin glucoside: >200 nmol g^{-1}FW	98

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Table 5. Continued

Target Nutrient/Crop	Gene Introduced/Modified	Promoter Used	Transgenic vs. WT	Reference
Stilbenes/Tomato	*STS*/V. vinifera *CHS*/Petunia + *CHR*/alfalfa *CHI*/petunia + *FNS-II*/gerbera	Pd35S	Stilbenes: 0.01mg g^{-1}FW (whole fruit); 0.037 mg g^{-1}FW (peel); deoxychalcones: 0.26 mg g^{-1}FW (peel) luteolin aglycon: 0.34 mg g^{-1}FW; luteolin 7-glucoside: 0.15 mg g^{-1}FW (peel); rutein: 0.90 vs. 56 mg g^{-1}FW (peel)	97
Resveratrol/Tomato	*StSy/V. vinifera*	CaMV35S	4-53 μg g^{-1}FW (nd in WT)	99
Phenolics and Stilbenes/Tomato	*StSy/V. vinifera*	CaMV35S	Caffeic acid: 0.058 mg g^{-1} FW; trans-piceid: 126.58 mg kg^{-1} FW; trans-resveratrol: 48.48 mg kg^{-1} FW	100
Piceid/Apple	*Vst1/V. vinifera*	Vst1(gene specific)	20-80 μg g^{-1}FW	101
Phenylpropanoids/Strawberry	*CHS*/Strawberry (antisense)	CaMV35S	100 vs. 1%	102
Tocopherols/Potato	*crtB*—TP/*E. uredovora*	Patatin (tuber specific)	α-tocopherol: 7.5 μg g^{-1} FW	72
	AtHPPD and *AtHPT*/Arabidopsis	CaMV 35S	α-tocopherol: (with *AtHPDD*) 338 vs. 432 ng g^{-1}FW; (with *AtHPT*) 578 vs. 284 ng g^{-1}FW	111
Fatty acyl composition/Canola	*CrtB*/bacteria	Napin	18:1 (oleic acid) component	69
Linolenic acid/Sweet potato	*NtFAD3*/Tobacco	E12Ω	22.1 vs. 10%	130

nd: not detected; DW: dry weight; FW: fresh weight.

constitutively as well as in a seed-specific manner in potato (Table 5).[62] The transgenic potato transformants accumulated protein and had increased levels of essential amino acids. The constitutive (16.12 mg/g tuber) as well as tuber-specific (16.58 mg/g tuber FW) expression of *AmA1* increased the total protein content of tuber to the same extent.

Methionine level was increased in alfalfa by introducing Arabidopsis cystathionine γ-synthase (*AtCGS*), the enzyme that controls the synthesis of the first metabolite in the methionine pathway, under the control of rubisco small subunit promoter.[63] This approach enhanced the methionine and cysteine contents in transgenics and the authors remarked that the 2-fold higher protein-bound methionine (3.65 ± 0.11 g methionine/100 g of protein) in transgenic alfalfa line L-1 amounts to an adequate supply of methionine in diet.

Genetically engineered accumulation of polyamines, spermidine and spermine, in tomato fruit resulted in the higher content of amino acids including asparagine, glutamate and glutamine, and choline.[23]

Carotenoids

Major Crops

Carotenoids have received considerable attention in recent years because of their antioxidant property as well as their potential in preventing malnutrition and disease. For instance, β-carotene is a pro-vitamin A and essential for vision. Lycopene is a potent antioxidant that has the potential to prevent epithelial cancers in addition to providing benefits to cardiovascular and immunity-based health. Rice is devoid of β-carotene. Therefore, genetic engineering was used to produce β-carotene in rice endosperm. Daffodil (*Narcissus pseudonarcissus*) *Psy* gene, which encodes phytoene synthase and produces the first carotenoid phytoene, a key precursor of β-carotene, was expressed in rice under a CaMV 35S (constitutive) or Gt1 (endosperm-specific) promoter. The transgenic rice accumulated 0.74 μg/g dry weight phytoene in rice seed while other carotenoids were not detected (Table 4).[64] This study catalyzed efforts to engineer the carotenoid biosynthetic pathway in rice endosperm using daffodil *Psy* gene and bacterial (*Erwinia uredevora*) phytoene desaturase *Crt1* gene.[54,65] Transgenic rice seeds were yellow in color due to the accumulation of carotenoids mainly β-carotene and, to some extent, lutein and zeaxanthin. Cotransformation with the vector carrying *psy* and *crt1* genes and that carrying daffodil lycopene β-cyclase gene led to segregating transgenic lines with an accumulation of carotenoid up to 1.6 μg/g.

Several studies have shown that *psy* gene product catalyzes a limiting and regulatory step in carotenoid biosynthesis. Several sources of *Psy* cDNA were used to transform rice in combination with the bacterial *crt1* gene expressed under the endosperm-specific promoter. The maximum carotenoid accumulation was achieved with maize *psy*, increasing the total carotenoid level to 37 μg/g, some 23-fold higher than a previous report on the Golden Rice.[55] Production of Golden Rice 2 is a landmark achievement and defuses the criticism that genetically modified rice is not good enough to fulfill the RDA of provitamin A in a normal daily diet.[65,66]

Bacterial (*Erwinia uredevora*) *crtB* and *crt1* genes were also expressed in white corn under the control of endosperm-specific duplicated zein promoter, which resulted in a color change in the kernels and a level of β-carotene that ranged from 7-10 μg/g dry seed.[67]

Minor Crops

Vegetables and fruits such as tomato and carrots are good sources of carotenoids, however the levels are below the RDA.[68,69] Transformation of canola seed with bacterial phytoene synthase (*crtB*) gene using seed-specific napin promoter resulted in orange colored seed with a 50-fold higher (1000-1500 μg/gFW) total carotenoid level than the wild type (33 μg/gFW) (Table 5).[70] Canola seeds coexpressing *crtI* and *crtB* gene accumulated lycopene (29 μg/gFW) and β-carotene (857 μg/gFW).[71] In a seed-specific manner, bacterial *crtB* gene expression in flaxseeds resulted in 7.8 to18.6-fold increase in the carotenoid levels.[72]

crtB gene was also used in a tuber-specific manner to increase carotenoids, violaxanthin, lutein and β-carotene, in transgenic potato.[73] A number of studies have focused on increasing the levels of

β-carotene in tomato (Table 5). Namely, fruit-specific[74,75] or constitutive[76] expression of lycopene β-cyclase and constitutive expression of phytoene desaturase[77] led to high levels of β-carotene in tomato fruit. The bacterial phytoene synthase fused to the tomato polygalacturonase promoter was introduced into tomato and the resulting transformants accumulated significantly higher levels of carotenoids, phytoene, lycopene, β-carotene and lutein.[78]

Transformation of tomato has also involved beneficial genes fused to regulatable promoters[79] or in using suppression RNAi to downregulate photomorphogenesis regulatory protein gene DE-ETIOLATED1 (*DET1*), a negative regulator of light mediated responses,[80] which showed the promise of biotechnology to obtain carotenoid levels high enough to meet the RDA dose. UV-DAMAGED DNA BINDING PROTEIN-1/high pigment-1 (*DDB1/HP1*) gene is a homologous gene of *DET1* that forms a complex with *Cul4* (Cullin 4, a component of Cul4 based E3 ligases, facilitates transfer of ubiquitin to the substrate for subsequent degradation by proteasome).[81,82] RNAi suppression of *CUL4* constitutively results in increased carotenoid accumulation.[83]

Downstream from the β-carotene pathway are important photoprotective compounds whose levels have also been successfully manipulated via genetic intervention. Using the fruit-specific Pds promoter to drive the expression of β-*Lcy* and β-*Chy* genes from Arabidopsis and pepper, respectively, about 100-fold increase in β-cryptoxanthin and zeaxanthin was achieved.[75]

Expression of yeast SAM dcarboxylase (*ySAMdc*) fused to a ripening-specific promoter E8 in tomato resulted in the accumulation of higher polyamines spermidine and spermine. The increase in the therapeutic polyamines in the transgenic lines signaled higher accumulation of other nutraceuticals such as lycopene, choline and various other health promoting phytonutrients.[2,84,85]

The cauliflower *Or* gene, *Orange* gene mutation responsible for β-carotene accumulation in cauliflower, when introduced in a tuber-specific manner into potato led to a 6-fold higher level of total carotenoids in transgenic versus the controls.[86,87] Ketocarotenoids are rare in plants but are strong antioxidants. They are chemically synthesized and used as dietary supplements and pigments in aquaculture and nutraceutical industry. Ketocarotenoid biosynthesis pathway was engineered in carrot by introducing an algal *Haematococcus pluvialis* β-carotene ketolase gene under the control of constitutive, ubiquitin or rolB promoter.[88] This resulted in 70% conversion of total β-carotene to ketocarotenoid that accumulated to a level of 2,400 μg/g root dry weight. These transgenic carrots have good potential for biopharming ketocarotenoid production for functional food, nutraceutical and aquaculture industries.

Folates

Major Crops

Plants are the main source of folates for human health. Many diseases are associated with or as a consequence of folate deficiency including neural tube defects, coronary and cardiovascular disease, Alzheimer's and a whole range of cancers and leukemia.[89]

Genetic enhancement of folates in plants is therefore seen as a major contribution to alleviate diseases associated with folate deficiency. Transgenic rice plants were generated with a construct consisting of Arabidopsis GTP cyclohydrolase I (GTPCH I) and aminodeoxychorismate synthase (ADCS) on a single T-DNA cassette under the control of rice endosperm-specific globulin (glb-1) and glutelin B1 (gluB1) promoters, respectively, to increase the folate content of rice seeds. The resulting transgenic seeds accumulated folates ranging from 6.0-38.3 nmol/g, respectively 15-100 times higher than the wild type. Interestingly, 89% of total folates in the transgenic rice was in the form of 5-methyltetrahydrate, which makes biofortified folates superior to folic acid-fortified rice.[90]

In the corn study, expression of *E. coli folE* gene encoding GTP cyclohydrolase (GCH1) under the control of the barley D-hordein promoter produced a 2-fold increase in folate content.[56]

Minor Crops

Folate enhancement in tomato was tested via engineering the GTP cyclohydrolase I (*gchI*) in pteridine, p-aminobenzoate (PABA) pathway.[91] Their strategy employed synthetic gene on

the basis of feedback-insensitive mammalian *gchI* and the fruit-specific E8 promoter to drive the expression. The transgenics accumulated twice as much folate as the nontransgenic control. The same laboratory also employed Arabidopsis aminodeoxychorismate synthase *ADCS* gene,[92] the first step in PABA biosynthesis to transform tomato, to increase further the folate levels. Upon crossing the gchI and ADCS lines to pyramid the two genes, the progeny obtained was found to accumulate 25-fold more folate than the controls. Such a remarkable enhancement in tomato folate level is at par with the RDA for adults.

Synthetic codon-optimized *gchI* based on native (*Gallus gallus*) chicken gene was introduced into lettuce to enhance folate levels. The transgenic lettuce lines thus generated had significantly increased folate content, which ranged from 2.1 to 8.5-fold higher than the nontransgenic lines.[93] The high-folate enriched lettuce would be sufficient to supply 26% of the RDA for folate, provided its bioavailability and bioactivity are optimal and equivalent.

Vitamin C (Ascorbate)

Major Crops

Dehydroascorbate reductase (DHAR) regenerates ascorbic acid (AsA) from its oxidized form and has been used to increase the level of AsA. Upon 100-fold increase in the expression of wheat *dhar* cDNA under the control of ubiquitin (ub) or Shrunken (Sh2) promoter in corn, 2 to 4-fold increase in AsA was quantified in kernels.[94] Expression of rice *dhar* (under the control of barley D-hordein promoter) in corn resulted in 6-fold increase in AsA (ascorbate).[56]

Minor Crops

Ascorbic acid levels were engineered in lettuce by constitutive expression of the rat AsA biosynthetic pathway gene, GulL oxidase; transgenic lettuce accumulated 4 to 7-fold higher AsA than the control plants.[95]

Polyphenolics

Major/Minor Crops

Polyphenols are also potent antioxidants and their potential as preventive for disease incidence in humans has catalyzed a number of studies to overaccumulate specific phenolics in crop plants. Most studies have employed key genes to transform vegetables and fruits. Constitutive over-expression of petunia chalcone isomerase *chi-a* gene, involved in flavonol biosynthesis, resulted in a 78-fold increase in flavonol content of tomato peel.[95] Chlorogenic acid and other flavonoids were also increased several-fold via constitutive expression of hydroxycinnamoyl transferase *HQT* gene.[97]

Plants are poor in flavones. However, genetic engineering has been able to produce novel polyphenolics/flavonoids in high concentrations in transgenic tomato fruit transformed with various, heterologous genes of flavonoid biosynthesis pathway.[98] Soybean isoflavone synthase (*IFS*) gene, whose protein product is a catalyst for the formation of genistein, was constitutively expressed in tomato. This resulted in the accumulation of isoflavone in leaves and fruit peels of transgenic tomato.[99] Similarly, introduction of grape stilbene synthase (*StSY*) gene in tomato generated resveratrol, which is not naturally present in tomato.[100] Transgenic fruit produced up to 53 μg/gFW of resveratrol which was accompanied with additional increases in the levels of ascorbate and glutathione. Trans-resveratrol, trans-piceid and cinnamic acid derivatives of flavonols and flavonones were identified by HPLC in the resveratrol-producing tomato transgenics.[100] When the same *StSy* gene was put under the control of its own wound, pathogen and UV inducible promoters and introduced into apple, the resultant transgenic apple synthesized phytoalexin resveratrol glucoside, piceid.[102]

In a different approach, constitutively expressed anti-sense strawberry chalcone synthase gene (*CHS*) led to the depletion of anthocyanin, flavonol and proanthocyanidines while phenylpropanoid pathway was upregulated.[103] Constitutive expression in potato of petunia dihydroflavonol

reductase gene (*DFR*), which encodes for a key enzyme in flavonoid biosynthesis, significantly enhanced anthocyanin and phenolic levels.[104]

Delila (*Del* encodes a basic helix-loop-helix transcription factor) and *Rosea1* (*Ros 1*-MYB-related transcription factor) genes from the snapdragon were fused to the ripening-specific E8 promoter and introduced into tomato.[105] Del and Ros1 are known to activate a broad spectrum of phenylpropanoid/flavonoid pathway genes in tomato. This approach resulted in high induction of anthocyanin accumulation in transgenic tomatoes.

Tocopherols

Major/Minor Crops

Tocopherols show anticarcinogenic properties and have the potential as adjuvant chemotherapeutics.[106] Several studies on enhancing the content of α-tocopherol, one of the most bioactive forms of tocopherol, have been reported. Over-expression of the *At-VTE4* (γ-*TMT*), which catalyzes the final step in the biosynthesis of α-tocopherol, in combination with *At VTE3* (2-methyl-6-phytylbenzoquinol methyltransferase) in soybean seed led to a 8-fold increase in α-tocopherol.[107] Subsequently, Arabidopsis γ-*TMT* was constitutively expressed in various crops, namely, soybean, oil seed and lettuce and in each case a higher level of α-tocopherol was obtained compared to the nontransgenic counterparts.[108-110] Similarly, *Perilla frutescens* γ-*TMT* introduced seed-specifically in soybean also produced higher levels of α-tocopherol and β-tocopherol.[111] In potato, tocopherol biosynthesis was manipulated by constitutively expressing *Arabidopsis* p-hydroxyphenylpyruvate dioxygenase (*At-HPPD*) or homogentisate phytyltransferase (*At-HPT*) genes, which led to respective accumulation of tuber α-tocopherol at 1030 ng/gFW and 579 ng/gFW compared to 281.5 ng/gFW in the controls.[112]

Iron

Major Crops

Rice endosperm is poor in iron content (7-24 mg/kg).[113] In an effort to improve the iron content of rice, soybean ferritin *pfe* gene was expressed under the control of seed storage protein promoter, glutelin GluB-1.[114] The iron content of transgenic rice seeds was three times (38.1 μg/gDW) as much as the wild type (14.3 μg/gDW) plants.

Phaseolus vulgaris ferritin *pfe* gene expressed under the control of Gt1 promoter also increased iron accumulation, varying from 11.53-22.07 μg/g rice seed, which is twice as much as in the wild type.[115] Following milling, wild type rice seeds contain low amounts of iron, 0.2-2.8 mg/100 g.[116] Expression of the soybean ferritin gene under the control of an endosperm-specific GluB-1 promoter led to higher amounts of iron and zinc in transgenic rice, each g of unpolished grain contained 71 μg of iron and 55.5 μg of zinc.[117]

Conclusion

Phytonutrients are now recognized as important determinants of human health. This has catalyzed investigations into broader aspects of plant-based nutraceuticals. These include: elucidation of biochemical pathways and identifying the rate-limiting steps; engineering metabolic pathways to direct the intermediary metabolism flux towards a particular nutrient (nutraceutical); testing efficacy of either an isolated and purified nutraceutical or a crop engineered with an enhanced nutrient level in animal and human models; testing crops silenced for health-detrimental factors including allergens; comparing bioavailability of an individual nutrient (nutraceutical) fed either as a food supplement or in the form of a fortified food. A common goal of such studies is to enable dietary intervention in human health to combat monogenic or polygenic diseases.

The molecular genetics and modern biotechnology approaches in conjunction with deciphering the metabolome of a crop plant are powerful tools that will help in specific redesigning of metabolism in food crops to accumulate desired, or close-to-the-desired, levels of a particular phytonutrient. Such studies will also help in driving higher production levels of nutraceuticals in

heterologous systems such as bacteria, algae and yeast. Crops can also be redesigned to be free of disease-causing food constituents. This should allow a neat treatment for patients suffering from monogenic, diet-related diseases, to name a few, CD, galactosemia, CMPA, glycogen storage disorder and phenylketonuria.

Progress in merging agriculture with preventive medicine will depend on intense collaborative research between physicians, nutritionists and plant biologists so that planning strategies are rationally designed and the developments in genetic technology successfully applied for better crop engineering. Engineering metabolism of plants can have a major impact of global proportions, in redesigning pathways to provide healthier and functional foods, improve the quality of raw materials and plant production factories to produce novel pharmaceuticals.

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