

Nutritionally Improved Agricultural Crops

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Agricultural innovation has always involved new, science-based products and processes that have contributed reliable methods for increasing productivity and sustainability. Biotechnology has introduced a new dimension to such innovation, offering efficient and cost-effective means to produce a diverse array of novel, value-added products and tools.

The first generation of biotechnology products commercialized were crops focusing largely on input agronomic traits whose value was largely opaque to consumers. The coming generations of crop plants can be grouped into four broad areas, each presenting what, on the surface, may appear as unique challenges to regulatory oversight. The present and future focus is on continuing improvement of agronomic traits such as yield and abiotic stress resistance in addition to the biotic stress tolerance of the present generation; crop plants as biomass feedstocks for biofuels and "biosynthetics"; value-added output traits such as improved nutrition and food functionality; and plants as production factories for therapeutics and industrial products. From a consumer perspective, the focus on value-added traits, especially improved nutrition, is of greatest interest.

Developing plants with these improved traits involves overcoming a variety of technical, regulatory, and indeed perception challenges inherent in the perceived and real challenges of complex modifications. Both traditional plant breeding and biotechnology-based techniques are needed to produce plants with the desired quality traits. Continuing improvements in molecular and genomic technologies are contributing to the acceleration of product development. Table I presents examples of crops that have already been genetically modified with macronutrient and micronutrient traits that may provide benefits to consumers and domestic animals.

NUTRITION VERSUS FUNCTIONALITY

At a fundamental level, food is viewed as a source of nutrition to meet daily requirements at a minimum in

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order to survive but with an ever greater focus on the desire to thrive. In the latter instance, there is an ever-growing interest in the functionality of food. Functional foods have been defined as any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains. The term nutraceutical is defined as "any substance that may be considered a food or part of a food and provides health benefits, including the prevention and treatment of disease" (Goldberg, 1994).

From the basic nutrition perspective, there is a clear dichotomy in demonstrated need between different regions and socioeconomic groups, the starkest being overconsumption in the developed world and undernourishment in less developed countries. Dramatic increases in the occurrence of obesity and related ailments in developed countries are in sharp contrast to the chronic malnutrition in many less developed countries. Both problems require a modified food supply, and the tools of biotechnology have a part to play. Worldwide, plant-based products comprise the vast majority of human food intake, irrespective of location or financial status (Mathers, 2006). In some cultures, either by design or default, plant-based nutrition actually comprises 100% of the diet. Therefore, it is to be expected that nutritional improvement can be achieved via modifications of staple crops.

While the correlative link between food and health is still open to debate, a growing body of evidence indicates that food components can influence physiological processes at all stages of life. Functional food components are of increasing interest in the prevention and/or treatment of at least four of the leading causes of death in the United States: cancer, diabetes, cardiovascular disease, and hypertension. The U.S. National Cancer Institute estimates that one in three cancer deaths are diet related and that eight of 10 cancers have a nutrition/diet component (Block et al., 1992; Potter and Steinmetz, 1996). Inverse relationships have been observed between carotenoid-rich foods and certain cancers (Botella-Pavía and Rodríguez-Concepción, 2006). Other nutrient-related correlations link dietary fat and fiber to the prevention of colon cancer, folate to the prevention of neural tube defects, calcium to the prevention of osteoporosis, psyllium to the lowering of blood lipid levels, and antioxidant nutrients to the scavenging of reactive oxidant species and protection against oxidative damage of cells that may lead to chronic disease, to list just a few

Table 1. Examples of crops in research and/or development with nutritionally improved traits intended to provide health benefits for consumers and animals

This list excludes protein/starch functionality, shelf life, taste/aesthetics, fiber quality, and allergen, antinutrient, and toxin reduction traits. Modified from International Life Sciences Institute (2004a, 2008).

Trait	Crop (Trait Detail)	Reference
Protein and amino acids		
Protein quality and level	Bahiagrass (protein↑)	Luciani et al. (2005)
	Canola (amino acid composition)	Roesler et al. (1997)
	Maize (amino acid composition; protein↑)	Cromwell et al. (1967, 1969), O'Quinn et al. (2000), Yang et al. (2002), Young et al. (2004)
	Potato (amino acid composition; protein↑)	Yu and Ao (1997), Chakraborty et al. (2000), Li et al. (2001), Atanassov et al. (2004)
	Rice (protein↑; amino acid composition)	Katsube et al. (1999)
	Soybean (amino acid balance)	Dinkins et al. (2001), Rapp (2002)
	Sweet potato (protein↑)	Egnin and Prakash (1997)
Essential amino acids	Canola (Lys↑)	Falco et al. (1995)
	Lupin (Met↑)	White et al. (2001)
	Maize (Lys↑, Met↑)	Lai and Messing (2002), Agbios (2008)
	Potato (Met↑)	Zeh et al. (2001)
	Sorghum (Lys↑)	Zhao et al. (2003)
	Soybean (Lys↑, Trp↑)	Falco et al. (1995), Galili et al. (2002)
Oils and fatty acids		
	Canola (lauric acid↑, GLA↑; + ω -3 fatty acids; 8:0 and 10:0 fatty acids↑; lauric and myristic acids↑; oleic acid↑)	Dehesh et al. (1996), Del Vecchio (1996), Roesler et al. (1997), Froman and Ursin (2002, 2003), James et al. (2003), Agbios (2008)
	Cotton (oleic acid↑, oleic + stearic acids↑)	Chapman et al. (2001), Liu et al. (2002)
	Grass, legumes (↓trans-fatty acids)	O'Neill (2007)
	Linseed (+ ω -3 and ω -6 fatty acids)	Abbadi et al. (2004)
	Maize (oil↑)	Young et al. (2004)
	Oil palm (oleic acid↑ or stearic acid↑, oleic acid↑, +palmitic acid↓)	Jalani et al. (1997), Parveez (2003)
	Rice (α -linolenic acid↑)	Anai et al. (2003)
	Soybean (oleic acid↑, GLA↑)	Reddy and Thomas (1996), Kinney and Knowlton (1998)
	Safflower (GLA↑)	Arcadia Biosciences (2008)
Carbohydrates		
Fructans	Chicory (fructan↑, fructan modification)	Smeekens (1997), Sprenger et al. (1997), Sévenier et al. (1998)
	Maize (fructan↑)	Caimi et al. (1996)
	Potato (fructan↑)	Hellwege et al. (1997)
	Sugar beet (fructan↑)	Smeekens (1997)
Fru, raffinose, stachyose	Soybean	Hartwig et al. (1997)
Inulin	Potato (inulin↑)	Hellwege et al. (2000)
Starch	Rice (amylase↑)	Schwall et al. (2000), Chiang et al. (2005)
Micronutrients and functional metabolites		
Vitamins and carotenoids	Canola (vitamin E↑)	Shintani and DellaPenna (1998)
	Maize (vitamin E↑, vitamin C↑)	Rochefford et al. (2002), Cahoon et al. (2003), Chen et al. (2003)
	Mustard (+ β -carotene)	Shewmaker et al. (1999)
	Potato (β -carotene and lutein↑)	Ducreux et al. (2005)
	Rice (+ β -carotene)	Ye et al. (2000)
	Strawberry (vitamin C↑)	Agius et al. (2003)
	Tomato (folate↑, phytoene and β -carotene↑, lycopene↑, provitamin A↑)	Rosati et al. (2000), Fraser et al. (2001), Mehta et al. (2002), Díaz de la Garza et al. (2004), Enfissi et al. (2005), DellaPenna (2007)
Functional secondary metabolites	Apple (+stilbenes)	Szankowski et al. (2003)
	Alfalfa (+resveratrol)	Hipskind and Paiva (2000)
	Kiwi (+resveratrol)	Kobayashi et al. (2000)
	Maize (flavonoids↑)	Yu et al. (2000)
	Potato (anthocyanin and alkaloid glycoside↓, solanin↓)	Lukaszewicz et al. (2004)
	Rice (flavonoids↑, +resveratrol)	Stark-Lorenzen et al. (1997), Shin et al. (2006)
	Soybean (flavonoids↑)	Yu et al. (2003)

(Table continues on following page.)

Table I. (Continued from previous page.)

Trait	Crop (Trait Detail)	Reference
Mineral availabilities	Tomato (+resveratrol, chlorogenic acid↑, flavonoids↑, stilbene↑)	Rosati et al. (2000), Muir et al. (2001), Niggeweg et al. (2004), Giovinazzo et al. (2005)
	Wheat (caffeic and ferulic acids↑, +resveratrol)	United Press International (2002)
	Alfalfa (phytase↑)	Austin-Phillips et al. (1999)
	Lettuce (iron↑)	Goto et al. (2000)
	Rice (iron↑)	Lucca et al. (2002)
	Maize (phytase↑, ferritin↑)	Drakakaki et al. (2005)
	Soybean (phytase↑)	Denbow et al. (1998)
	Wheat (phytase↑)	Brinch-Pedersen et al. (2000)

(Mutch et al., 2005; Mathers, 2006). Many food components are known to influence the expression of both structural genes and transcription factors (Tfs) in humans (Go et al., 2005; Mazzatti et al., 2007). Examples of these phytochemicals are listed in Table II. The large diversity of phytochemicals suggests that the potential impact of phytochemicals and functional foods on human and animal health is worth examining as targets of biotechnology efforts.

On the functionality side, there is a mirror component from the perspective of the genetic makeup of the individual doing the consuming. This field of personal response to nutrients is further divided into two thematic subsets with subtle differences. Nutrigenomics is the prospective analysis of differences among nutrients in the regulation of gene expression, while nutrigenetics is the analysis of genetic variations among individuals with respect to the interaction between diet and disease. These spheres of enquiry are designed to provide nutritional recommendations for personalized or individualized nutrition (Brigelius-Flohe and Joost, 2006). Haplotyping studies are beginning to indicate gender- and ethnicity-specific polymorphisms that are implicated in susceptibilities to polygenic disorders such as diabetes, cardiovascular disease, and some cancers (Corthésy-Theulaz et al., 2005; Mutch et al., 2005; Brigelius-Flohe and Joost, 2006). For example, several studies have reported some evidence to suggest that the risks from high intake of well-done meat are higher in fast or presumed fast acetylator haplotypes (NAT1 and/or NAT2) or in rapid NAT2 (haplotypes) and CYP1A2 phenotypes. During cooking of muscle meat at high temperature, some amino acids may react with creatine to give heterocyclic aromatic amines. Heterocyclic aromatic amines can be activated through acetylation to reactive metabolites, which bind DNA and cause cancers. Only NAT2 fast acetylators can perform this acetylation. Studies have shown that the NAT2 fast acetylator genotype had a higher risk of developing colon cancer in people who consumed relatively large quantities of red meat. Understanding individual response is at least as complex a challenge as the task of increasing or decreasing the amount of a specific protein, fatty acid, or other component of the plant itself (Brigelius-Flohe and Joost, 2006). It is of little use

producing a plant with a supposed nutritional benefit unless that benefit actually improves the health of humans or animals.

From a health perspective, plant components of dietary interest can be broadly divided into four main categories, the first two to be enhanced and the latter two to be limited or removed: macronutrients (proteins, carbohydrates, lipids [oils], fiber); micronutrients (vitamins, minerals, functional metabolites); antinutrients (substances such as phytate that limit the bioavailability of nutrients); and allergens (intolerances and toxins).

THE TECHNOLOGY

As noted, plants are a treasure trove of interesting and valuable compounds, since they must glean everything from the spot on earth where they are rooted and they cannot escape when threatened; therefore, they have evolved a most impressive panoply of products to thrive in ever-changing environments despite these limitations. It is estimated that plants produce up to 200,000 phytochemicals across their many and diverse members (Oksman-Caldenty and Inzé, 2004); obviously, a more truncated subset of this number is available on our food palate, with approximately 25,000 different metabolites in general plant foods (Go et al., 2005). The quality of crop plants, nutritionally or otherwise, is a direct function of this metabolite content (Memelink, 2004). This brings metabolomic approaches front and center both in better understanding what has occurred during crop domestication (lost and silenced traits) and in designing new paradigms for more targeted crop improvement that are better tailored to current needs (Hall et al., 2008). In addition, of course, with modern techniques we have the potential to trawl the rest of that biochemical treasure trove to find and introgress traits of value that were outside the scope of previous breeding strategies.

Research to improve the nutritional quality of plants has historically been limited by a lack of basic knowledge of plant metabolism and the compounding challenge of resolving the complex interactions of thousands of metabolic pathways. Both traditional plant breeding and biotechnology techniques are needed to metabolically engineer plants with desired quality

Table II. Examples of plant components with suggested functionality

The examples shown do not constitute an all-inclusive list. Modified from International Life Sciences Institute (2004a).

Class/Component	Source	Potential Health Benefit
Carotenoids		
α -Carotene	Carrot	Neutralizes free radicals that may cause damage to cells
β -Carotene	Various fruits, vegetables	Neutralizes free radicals
Lutein	Green vegetables	Contributes to maintenance of healthy vision
Lycopene	Tomato and tomato products (ketchup, sauces)	May reduce risk of prostate cancer
Zeaxanthin	Eggs, citrus, maize	Contributes to maintenance of healthy vision
Dietary fiber		
Insoluble fiber	Wheat bran	May reduce risk of breast and/or colon cancer
β -Glucan ^a	Oat	May reduce risk of cardiovascular disease (CVD)
Soluble fiber ^a	Psyllium	May reduce risk of CVD
Whole grains ^a	Cereal grains	May reduce risk of CVD
Collagen hydrolysate	Gelatin	May help improve some symptoms associated with osteoarthritis
Fatty acids		
ω -3 fatty acids (DHA/EPA)	Tuna fish and marine oils	May reduce risk of CVD and improve mental and visual functions
Conjugated linoleic acid	Cheese, meat products	May improve body composition, may decrease risk of certain cancers
GLA	Borage, evening primrose	May reduce inflammation risk of cancer and CVD, may improve body composition
Flavonoids		
Anthocyanidins: cyanidin	Berries	Neutralize free radicals, may reduce risk of cancer
Hydroxycinnamates	Wheat	Antioxidant-like activities, may reduce risk of degenerative diseases
Flavanols: catechins, tannins	Tea (green, catechins; black, tannins)	Neutralize free radicals, may reduce risk of cancer
Flavanones	Citrus	Neutralize free radicals, may reduce risk of cancer
Flavones: quercetin	Fruits/vegetables	Neutralize free radicals, may reduce risk of cancer
Glucosinolates, indoles, isothiocyanates		
Sulforaphane	Cruciferous vegetables (broccoli, kale), horseradish	Neutralizes free radicals, may reduce risk of cancer
Phenolics		
Stilbenes (resveratrol)	Grape	May reduce risk of degenerative diseases, heart disease, and cancer, may have longevity effect
Caffeic acid, ferulic acid	Fruits, vegetables, citrus	Antioxidant-like activities, may reduce risk of degenerative diseases, heart disease, and eye disease
Epicatechin	Cacao	Antioxidant-like activities, may reduce risk of degenerative diseases and heart disease
Plant stanols/sterols		
Stanol/sterol ester ^a	Maize, soy, wheat, wood oils	May reduce risk of coronary heart disease by lowering blood cholesterol levels
Prebiotic/probiotics		
Fructans, inulins, fructo-oligosaccharides	Jerusalem artichoke, shallot, onion powder	May improve gastrointestinal health
<i>Lactobacillus</i>	Yogurt, other dairy	May improve gastrointestinal health
Saponins	Soybean, soy foods, soy protein-containing foods	May lower LDL cholesterol, contains anticancer enzymes
Soybean protein	Soybean and soy-based foods	25 g d ⁻¹ may reduce risk of heart disease
Phytoestrogens		
Isoflavones (daidzein, genistein)	Soybean and soy-based foods	May reduce menopause symptoms, such as hot flashes, may reduce osteoporosis and CVD
Lignans	Flax, rye, vegetables	May protect against heart disease and some cancers, may lower LDL cholesterol, total cholesterol, and triglycerides
Sulfides/thiols		
Diallyl sulfide	Onion, garlic, olive, leek, scallion	May lower LDL cholesterol, helps to maintain healthy immune system
Allyl methyl trisulfide, dithiolthiones	Cruciferous vegetables	May lower LDL cholesterol, helps to maintain healthy immune system
Tannins		
Proanthocyanidins	Cranberry, cranberry products, cocoa, chocolate, black tea	May improve urinary tract health, may reduce risk of CVD and high blood pressure

^aU.S. Food and Drug Administration-approved health claim established for component.

traits. Metabolic engineering is generally defined as the redirection of one or more enzymatic reactions to improve the production of existing compounds, produce new compounds, or mediate the degradation of undesirable compounds. It involves the redirection of cellular activities by the modification of the enzymatic, transport, and regulatory functions of the cell. Significant progress has been made in recent years in the molecular dissection of many plant pathways and in the use of cloned genes to engineer plant metabolism.

With the tools now being harnessed through the many “omics” and “informatics” fields, there is the potential to identify genes of value across species, phyla, and kingdoms. Through advances in proteomics and glycomics, we are beginning to quantify simultaneously the levels of many individual proteins and to follow posttranslational alterations that occur in pathways. Ever more sophisticated metabolomic tools and analysis systems allow the study of both primary and secondary metabolic pathways in an integrated fashion (Hall et al., 2008). However, the increasing sophistication of this tool also demonstrates some anomalies in relying on this approach. For example, in potato (*Solanum tuberosum*), flow injection mass spectrometry analysis of a range of genotypes revealed genotypic correlations with quality traits such as free amino acid content (Beckman et al., 2007). Yet, matrix-assisted laser desorption/ionization chemotyping and gas chromatography-mass spectrometry profiling of tomato (*Solanum lycopersicum*) cultivars have revealed extensive differences in metabolic composition (sugars, amino acids, organic acids) despite close specific/genotypic similarities (Carrari et al., 2006; Fraser et al., 2007). Likewise, with regard to metabolomic analysis on the consumer side, little is known of the extent to which changes in the nutrient content of the human diet elicit changes in metabolic profiles. Moreover, the metabolomic signal from nutrients absorbed from the diet must compete with myriad nonnutrient signals that are absorbed, metabolized, and secreted.

Although progress in dissecting metabolic pathways and our ability to manipulate gene expression in genetically modified (GM) plants has progressed apace, attempts to use these tools to engineer plant metabolism have not quite kept pace. Since the success of this approach hinges on the ability to change host metabolism, its continued development will depend critically on a far more sophisticated knowledge of plant metabolism, especially the nuances of interconnected cellular networks, than currently exists. This complex interconnectivity is regularly demonstrated. Relatively minor genomic changes (point mutations, single gene insertions) are regularly observed following metabolomic analysis, leading to significant changes in biochemical composition (Bino et al., 2005; Long et al., 2006; Davidovich-Rikanati et al., 2007). Giliberto et al. (2005) used a genetic modification approach to study the mechanism of light influence on antioxidant content (anthocyanin, lycopene) in the tomato cv Money-maker. However, other, what on the surface would

appear to be more significant, genetic changes unexpectedly yield little phenotypic effect (Schauer and Fernie, 2006).

Likewise, there are unexpected outcomes, such as the fact that significant modifications made to primary Calvin cycle enzymes (Fru-1,6-bisphosphatase and phosphoribulokinase) have little effect while modifications to minor enzymes (e.g. aldase, which catalyzes a reversible reaction) seemingly irrelevant to pathway flux have major effects (Hajirezaei et al., 1994; Paul et al., 1995). These observations drive home the point that a thorough understanding of the individual kinetic properties of enzymes may not be informative as to their role (Haake et al., 1998). They also make clear that caution must be exercised when extrapolating individual enzyme kinetics to the control of flux in complex metabolic pathways. With these evolving tools, a better understanding of the global effects of metabolic engineering on metabolites, enzyme activities, and fluxes is beginning to be developed. Attempts to modify storage proteins or secondary metabolic pathways have also been more successful than have alterations of primary and intermediary metabolism (DellaPenna and Pogson, 2006). While offering great opportunities, this plasticity in metabolism complicates potential routes to the design of new, improved crop varieties. Regulatory oversight of engineered products has been designed to detect such unexpected outcomes in biotech crops, and as demonstrated by Chassy et al. (International Life Sciences Institute, 2004a, 2004b, 2008), existing analytical and regulatory systems are adequate to address novel metabolic modifications in nutritionally improved crops.

One potential approach to counter some of the complex problems in the metabolic engineering of pathways involves the manipulation of Tfs that control networks of metabolism (Kinney and Knowlton, 1998; Bruce et al., 2000). For example, expression of the maize (*Zea mays*) Tfs C1 and R, which regulate the production of flavonoids in maize aleurone layers under the control of a strong promoter, resulted in a high accumulation rate of anthocyanins in *Arabidopsis* (*Arabidopsis thaliana*), presumably by activating the entire pathway (Bruce et al., 2000). DellaPenna (Welsch et al., 2007) found that Tf RAP2.2 and its interacting partner SINAT2 increased carotenogenesis in *Arabidopsis* leaves. Expressing the Tf Dof1 induced the up-regulation of genes encoding enzymes for carbon skeleton production, a marked increase of amino acid content, and a reduction of the Glc level in transgenic *Arabidopsis* (Yanagisawa, 2004), and the DOF Tf AtDof1.1 (OBP2) up-regulated all steps in the glucosinolate biosynthetic pathway in *Arabidopsis* (Skirycz et al., 2006). Such expression experiments hold promise as an effective tool for the determination of transcriptional regulatory networks for important biochemical pathways. In summary, metabolic engineers must not only understand the fundamental physiology of the process to be affected but also the level, timing, sub-cellular location, and tissue or organ specificity that will

be required to ensure successful trait modification. Gene expression can be modulated by numerous transcriptional and posttranscriptional processes. Correctly choreographing these many variables is the factor that makes metabolic engineering in plants so challenging.

As a corollary to these techniques, there are several new technologies that can overcome the limitation of single gene transfers and facilitate the concomitant transfer of multiple components of metabolic pathways. One example is multiple transgene direct DNA transfer, which simultaneously introduces all of the components required for the expression of complex recombinant macromolecules into the plant genome, as demonstrated by Nicholson et al. (2005), who successfully delivered into rice (*Oryza sativa*) plants four transgenes that represent the components of a secretory antibody. More recently, Carlson et al. (2007) constructed a minichromosome vector that remains autonomous from the plant's chromosomes and stably replicates when introduced into maize cells. This work makes it possible to design minichromosomes that carry cassettes of genes, enhancing the ability to engineer plant processes such as the production of complex biochemicals. Christou and Kohli (2005) demonstrated that gene transfer using minimal cassettes is an efficient and rapid method for the production of transgenic plants containing and stably expressing several different transgenes. Since no vector backbones are required, thus preventing the integration of potentially recombinogenic sequences, they remain stable across generations. These groups' constructions facilitate the effective manipulation of multigene pathways in plants in a single transformation step, effectively recapitulating the bacterial operon model in plants. More recently, Christou and colleagues (Agrawal et al., 2005; Christou and Kohli, 2005) demonstrated this principle by engineering the entire carotenoid pathway in white maize, visually creating a latter day rainbow equivalent of Indian maize depending on the integrated transgene complement. This system has an added advantage from a commercial perspective in that these methods circumvent problems with traditional approaches that not only limit the amount of sequences transferred but may disrupt native genes or lead to poor expression of the transgene, thus reducing both the numbers of transgenic plants that must be screened and the subsequent breeding and introgression steps required to select a suitable commercial candidate.

The agronomically improved GM crops now being grown on more than 114 million ha around the world are products of the application of these technologies to crop plants (James, 2008). They generally involve the relatively simple task of adding a single gene or a small number of genes to plants. These genes in the main function outside of the plant's primary metabolic processes and thus have little or no effect on the composition of the plants. In addition to numerous success stories, some studies, as noted, even with these simpler modifications, have yielded unanticipated re-

sults. For example, the concept of gene silencing emerged from the unexpected observation that adding a chalcone synthase gene to increase color in *Petunia* spp. resulted instead in the switching off of color, producing white and variegated flowers (Napoli et al., 1990). This initially unexpected observation, now termed RNA interference, is one of the principal tools applied in everything from the analysis of molecular evolution to designing targeted therapeutics. In plants, it has now been turned to advantage in the first generation, developing robust virus resistance through coat protein posttranscriptional gene silencing, and in nutritional improvement, such as switching off of expression of an allergen in soybean (*Glycine max*).

To summarize, omics-based strategies for gene and metabolite discovery, coupled with high-throughput transformation processes and automated analytical and functionality assays, have accelerated the identification of product candidates. Identifying rate-limiting steps in synthesis could provide targets for genetically engineering biochemical pathways to produce augmented amounts of compounds and new compounds. Targeted expression will be used to channel metabolic flow into new pathways, while gene-silencing tools can reduce or eliminate undesirable compounds or traits or switch off genes to increase desirable products (Liu et al., 2002; Herman et al., 2003; Davies, 2007). In addition, molecular marker-based breeding strategies have already been used to accelerate the process of introgressing trait genes into high-yielding germplasm for commercialization. In the interest of space, Table I summarizes the work done to date on specific applications in the categories listed above. The following sections provide brief examples of some specific applications under those categories.

MACRONUTRIENTS

Protein

Protein energy malnutrition is the most lethal form (Food and Agriculture Organization, 2006) of malnutrition and affects every fourth child worldwide, according to the World Health Organization (2006). The Food and Agriculture Organization estimates that 850 million people worldwide suffer from undernutrition, to which insufficient protein in the diet is a significant contributing factor. Most plants have a poor balance of essential amino acids relative to the needs of animals and humans. The cereals (maize, wheat [*Triticum aestivum*], rice, etc.) tend to be low in Lys, whereas legumes (soybean, pea [*Pisum sativum*]) are often low in the sulfur-rich amino acids Met and Cys. Poultry, swine, and other nonruminant animals have specific requirements for each of the essential amino acids. The primary requirements for maize and soybean meal-based diets are Lys in mammals and Met in avian species. High-Lys and high-Met maize and soybeans could allow diet formulations that reduce animal nitrogen excretion by providing an improved

balance of essential amino acids. When they are out of balance, the amino acid in excess results in increased nitrogen excretion. That balance can be accomplished now, but only by adding costly synthetic Lys and Met to the diet. Successful examples of improving amino acid balance to date include high-Lys maize (Eggeling et al., 1998; O'Quinn et al., 2000) canola (*Brassica napus*), and soybean (Falco et al., 1995). Free Lys is significantly increased in high-Lys maize by the introduction of the *dapA* gene from *Corynebacterium glutamicum*, which encodes a form of dihydrodipicolinate synthase that is insensitive to Lys feedback inhibition. As a cautionary tale in this successful system, substantial increases in Lys only occurred in plants in which flux increased to such a level that the first enzyme of the catabolic pathway became saturated (Brinch-Pedersen et al., 2000), again illustrating the potential complexities of metabolic regulation. Consumption of foods made from these crops potentially can help to prevent malnutrition in developing countries, especially among children.

Another method of modifying storage protein composition is to introduce heterologous or homologous genes that code for proteins containing elevated levels of the desired amino acid, such as sulfur-containing Met and Cys or Lys. An interesting solution to this is to create a completely artificial protein containing the maximum number of the essential amino acids Met, Thr, Lys, and Leu in a stable, helical conformation designed to resist proteases to prevent degradation. This was done by Beauregard et al. (1995), who created an 11-kD synthetic protein, MBI, with 16% Met and 12% Lys, which they introduced into soybean using vectors targeted to seed protein storage bodies using appropriate leader sequences and seed-specific promoters (Simmonds and Donaldson, 2000). This was also achieved in a nonseed food crop, sweet potato (*Ipomoea batatas*), modified with an artificial storage protein gene (Egnin and Prakash, 1997). These transgenic plants exhibited 2- and 5-fold increases in the total protein content in leaves and roots, respectively, over that of control plants. A significant increase in the level of essential amino acids, such as Met, Thr, Trp, Ile, and Lys, was also observed (Egnin and Prakash, 1997; International Life Sciences Institute, 2008). A key issue is to ensure that the total amount and composition of storage proteins is not altered to the detriment of the development of the crop plant when attempting to improve amino acid ratios (Rapp, 2002). Since this is a completely novel protein to the human diet, it will be subjected to extensive review; yet, as demonstrated by the International Life Sciences Institute (2008), the existing regulatory and analytic methods are appropriate and sufficient to achieve this aim.

Young et al. (2004) used a novel approach to indirectly increase protein and oil content. They used a bacterial cytokinin-synthesizing isopentenyl transferase enzyme, under the control of a self-limiting senescence-inducible promoter, to block the loss of the lower floret, resulting in the production of just one

kernel composed of a fused endosperm with two viable embryos. The presence of two embryos in a normal-sized kernel leads to the displacement of endosperm growth, resulting in kernels with an increased ratio of embryo to endosperm content. The end result is maize with more protein and oil and less carbohydrate (Young et al., 2004; International Life Sciences Institute, 2008).

Carbohydrates

As the somewhat disputed notion of a glycemic index has supplanted Atkins as the indicator of choice when addressing carbohydrates in the diet, it has become clear to the public that not all carbohydrates are created equal. While it is still something of a value judgment to describe "good" versus "bad" carbohydrates, there are clear clinical indications of the value of polymeric versus simple sugars. Plants are effective at making both polymeric carbohydrates (e.g. starches and fructans) and individual sugars (e.g. Suc and Fru). The biosynthesis of these compounds is sufficiently understood to allow the bioengineering of their properties or to engineer crops to produce polysaccharides not normally present.

Fructans are an important ingredient in functional foods because evidence suggests that they promote a healthy colon (as a prebiotic agent) and help reduce the incidence of colon cancer. Sévenier et al. (1998) reported high-level fructan accumulation in a transgenic sugar beet (*Beta vulgaris*) without adverse effects on growth or phenotype. This work has implications both for the commercial manufacture of fructans and also for the use of genetic engineering to obtain new products from existing crops. Fructans consisting of linear β -(1 \rightarrow 2)-linked Fru polymers are called inulins. Hellwege et al. (2000) produced a transgenic potato synthesizing the full spectrum of inulin molecules naturally occurring in globe artichoke (*Cynara scolymus*) roots. A similar approach is being used to derive soybean varieties that contain some oligofructan components that selectively increase the population of beneficial species of bacteria (e.g. bifidobacteria) in the intestines of humans and certain animals and inhibit the growth of harmful species of bacteria (e.g. *Escherichia coli* 0157:H7, *Salmonella* spp., etc.; Bouhnik et al., 1999) When colonic bacteria ferment dietary fiber or other unabsorbed carbohydrates, the products are short-chain saturated fatty acids. These short-chain fatty acids may enhance the absorption of minerals such as iron, calcium, and zinc; induce apoptosis, preventing colon cancer; and inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, thus lowering low-density lipoprotein (LDL) production (Watkins et al., 1999; German et al., 2005).

The amylose-amylopectin ratio has the greatest influence on the physicochemical properties of the starch, and for many applications it is desirable to have a pure or enriched fraction of either amylopectin or amylose. Schwall et al. (2000) created a potato

producing very high-amylose (slowly digested) starch by inhibiting two enzymes that would normally make the amylopectin type of starch that is rapidly digested. This "resistant starch" is not digested in the small intestine but is fermented in the large intestine by microflora. Clinical studies have demonstrated that resistant starch has similar properties to fiber and has potential physiological benefits in humans (Yue and Waring, 1998; Richardson et al., 2000).

Fiber

Fiber is a group of substances chemically similar to carbohydrates, but nonruminant animals including humans poorly metabolize fiber for energy or other nutritional uses. Fiber is only found in foods derived from plants and never occurs in animal products. Fiber provides bulk in the diet, such that foods rich in fiber offer satiety without contributing significant calories. Current controversies aside, there is ample scientific evidence to show that prolonged intake of dietary fiber has various positive health benefits, especially the potential for reduced risk of colon and other types of cancer. Fiber type and quantity are undoubtedly under genetic control, although this topic has been little studied. The technology to manipulate fiber content and type by genetic engineering would be a great benefit to the health status of many individuals who refuse, for taste or other reasons, to include adequate amounts of fiber in their daily diet. For example, fiber content could be added to more preferred foods or the more common sources of dietary fiber could be altered for greater health benefits.

Nonruminant animals do not produce enzymes necessary to digest cellulose-based plant fiber. Plants low in fiber should yield more digestible and metabolizable energy and protein and less manure and methane when fed to monogastric species (North Carolina Cooperative Extension Service, 2000). Vermerris and Bout (2003) cloned a *Brown midrib* gene that encodes caffeic acid *O*-methyltransferase, a lignin-producing enzyme. They generated mutants that give rise to plants that contain significantly lower lignin in their leaves and stems, leading to softer cell walls compared with the wild type. The plant-softening mutations improve the digestibility of the food, and livestock also seem to prefer the taste. Such improved fiber digestibility in ruminants should have significant beneficial effects, because the efficiency of digestion of most high-fiber diets for ruminants is far from optimized.

Novel Lipids

Gene technology and plant breeding are combining to provide powerful means for modifying the composition of oilseeds to improve their nutritional value and provide the functional properties required for various food oil applications. Genetic modification of oilseed crops can provide an abundant, relatively inexpensive source of dietary fatty acids with wide-ranging health

benefits. Production of such lipids in vegetable oil provides a convenient mechanism to deliver healthier products to consumers without the requirement for significant dietary changes. Major alterations in the proportions of individual fatty acids have been achieved in a range of oilseeds using conventional selection, induced mutation, and, more recently, posttranscriptional gene silencing. Examples of such modified oils include low- and zero-saturated fat soybean and canola oils, canola oil containing medium-chain fatty acids, high-stearic acid canola oil (for trans-fatty acid-free products), high-oleic acid (monounsaturated) soybean oil, and canola oil containing the polyunsaturated fatty acids γ -linolenic acid (GLA; 18:3 n-6) and stearidonic acid (SDA; C18:4 n-3), very-long-chain fatty acids (Zou et al., 1997), and ω -3 fatty acids (Yuan and Knauf, 1997). Many of these modified oils are being marketed, and a number of countries have a regulatory system in place for the premarket safety review of novel foods produced through conventional technology.

Medium chain fatty acids range from 6 to 10 carbons long and are only minor components of natural foods, with the exception of coconut and palm kernel oils. When medium-chain triglycerides (MCTs) are substituted for long-chain triglycerides (LCTs) in the diet, animals gain less weight, store less adipose tissue, and experience an increase in metabolic rate (Baba et al., 1982; Geliebter et al., 1983). Mice fed diets with MCTs have also been shown to possess increased endurance in swimming tests over that of mice fed diets with LCTs (Fushiki et al., 1995). Expression of an acyl-ACP thioesterase cDNA from *Cuphea hookeriana* in seeds of transgenic canola, an oilseed crop that normally does not accumulate any capric and caprylic acids, resulted in a dramatic increase in the levels of these two MCTs (Dehesh et al., 1996).

Edible oils rich in monounsaturated fatty acids provide improved oil stability, flavor, and nutrition for human and animal consumption. Oleic acid (18:1), a monounsaturate, can provide more stability than the polyunsaturates linoleic acid (18:2) and linolenic acid (18:3). From a health aspect, the monounsaturates are also preferred. Antisense inhibition of oleate desaturase expression in soybean resulted in oil that contained more than 80% oleic acid (23% is normal) and had a significant decrease in polyunsaturated fatty acids (Kinney and Knowlton, 1998). High-oleic-acid soybean oil is naturally more resistant to degradation by heat and oxidation and so requires little or no postrefining processing (hydrogenation), depending on the intended vegetable oil application. In 2009, DuPont hopes to introduce soybean oil composed of at least 80% oleic acid, linolenic acid of about 3%, and over 20% less saturated fatty acids than commodity soybean oil. Monsanto's Vistive contains less than 3% linolenic acid, compared with 8% for traditional soybeans. These result in more stable soybean oil and less need for hydrogenation and concomitant reduction in trans-fatty acids. For lower trans-fats in livestock products, Nicholas Roberts and Richard Scott at AgResearch (New Zealand) are

researching an ingenious method to prevent plant-derived cis-polyunsaturated fatty acids from being transformed into saturated trans-fats in the rumen by borrowing an adaptation from plants themselves. They are engineering forage crops, such as grasses and legumes, with polyoleosin genes from sesame (*Sesamum indicum*), which should result in triglycerides being encapsulated within self-assembling polyoleosin micelles, thus sealing them off from bacterial activity during transit through the rumen (O'Neill, 2007).

A key function of α -linolenic acid is as a substrate for the synthesis of longer chain ω -3 fatty acids found in fish, eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), which play an important role in the regulation of inflammatory immune reactions and blood pressure, brain development in utero, and, in early postnatal life, the development of cognitive function. SDA (C18:4n-3), EPA, and DHA also possess anticancer properties (Christensen et al., 1999; Smuts et al., 2003; Reiffel and McDonald, 2006). Research indicates that the ratio of n-3 to n-6 fatty acids may be as important to health and nutrition as the absolute amounts present in the diet or in body tissues. Current western diets tend to be relatively high in n-6 fatty acids and relatively low in n-3 fatty acids. Production of a readily available source of long-chain polyunsaturated fatty acids, specifically ω -3 fatty acids, delivered in widely consumed prepared foods could deliver much needed ω -3 fatty acids to large sectors of the population with skewed n-6:n-3 ratios. In plants, the microsomal ω -6 desaturase-catalyzed pathway is the primary route of production of polyunsaturated lipids. Ursin (2003) has introduced the Δ -6 desaturase gene from a fungus (*Mortierella*), succeeding in producing ω -3 in canola. In a clinical study, James et al. (2003) observed that SDA was superior to α -linolenic acid as a precursor by a factor of 3.6 in producing EPA, DHA, and docosapentaenoic acid (C22:5n-3). Transgenic canola oil was obtained that contains more than 23% SDA, with an overall n-6:n-3 ratio of 0.5.

However, not all ω -6 fatty acids are created equal. GLA (C18:3n-6) is an ω -6 fatty acid with health benefits that are similar and complementary to the benefits of ω -3 fatty acids, including anti-inflammatory effects, improved skin health, and weight loss maintenance (Schirmer and Phinney, 2007). Arcadia Biosciences (2008) has engineered GLA safflower oil, with up to 40% GLA, essentially quadrupling the levels obtained in source plants such as evening primrose (*Oenothera biennis*) and borage (*Borago officinalis*).

Structural lipids also have positive health benefits; for example, in addition to their effect in lowering cholesterol, membrane lipid phytosterols have been found to inhibit the proliferation of cancer cells by inducing apoptosis and G1/S cell cycle arrest through the 3-hydroxy-3-methylglutaryl-CoA reductase target mentioned previously (Awad and Fink, 2000). In addition to this and the above, specialty oils may also be developed with further pharmaceutical and chemical feedstock applications in mind.

Vitamins and Minerals

Micronutrient malnutrition, the so-called hidden hunger, affects more than half of the world's population, especially women and preschool children in developing countries (United Nations System Standing Committee on Nutrition, 2004). Even mild levels of micronutrient malnutrition may damage cognitive development, lower disease resistance in children, and increase the incidence of childbirth mortality. The costs of these deficiencies, in terms of diminished quality of life and lives lost, are enormous (Pfeiffer and McClafferty, 2007). The clinical and epidemiological evidence is clear that select minerals (iron, calcium, selenium, and iodine) and a limited number of vitamins (folate, vitamins E, B6, and A) play a significant role in the maintenance of optimal health and are limiting in diets.

Using various approaches, vitamin E levels are being increased in several crops, including soybean, maize, and canola, while rice varieties are being developed with the enhanced vitamin A precursor, β -carotene, to address vitamin A deficiency that leads to macular degeneration and affects development. Ameliorating another major deficiency in less developed countries, minerals such as iron and zinc have also been addressed. Other targets include improved iron content, ferritin-rich lettuce (*Lactuca sativa*), bioavailable phosphorus, and divalent ions released from phytate, folate-enriched tomatoes, and isoflavonoids (DellaPenna, 2007; Yonekura-Sakakibara et al., 2007).

As with macronutrients, one way to ensure an adequate dietary intake of nutritionally beneficial phytochemicals is to adjust their levels in plant foods. Until recently, such work had been hindered by the difficulty of isolating the relevant genes (e.g. for vitamin biosynthesis). However, the advent of genomics during the past few years has provided new routes for such work. Using nutritional genomics, DellaPenna (Shintani and DellaPenna, 1998; DellaPenna, 2007) isolated a gene, γ -tocopherol methyltransferase, that converts the lower activity precursors to the highest activity vitamin E compound, α -tocopherol. With this technology, the vitamin E content of Arabidopsis seed oil has been increased nearly 10-fold and progress has been made to move the technology to agricultural crops such as soybean, maize, and canola (DellaPenna, 2007).

Rice is a staple that feeds nearly half the world's population, but milled rice does not contain any β -carotene or its carotenoid precursors. Integrating observations from prokaryotic systems into their work enabled researchers to clone the majority of carotenoid biosynthetic enzymes from plants during the 1990s. Taking advantage of this, Golden rice, with β -carotene expression in the endosperm, was created (Ye et al., 2000). The health benefits of the original Golden rice and, especially, Golden rice II are well reviewed (International Life Sciences Institute, 2008). A similar method was used by Monsanto to produce β -carotene in canola.

Iron is the most commonly deficient micronutrient in the human diet, and iron deficiency affects an estimated 1 to 2 billion people. Anemia, characterized by low hemoglobin, is the most widely recognized symptom of iron deficiency, but there are other serious problems, such as impaired learning ability in children, increased susceptibility to infection, and reduced work capacity. A research group led by Goto et al. (2000) and another led by Lucca et al. (2002) employed the gene for ferritin, an iron-rich soybean storage protein, under the control of an endosperm-specific promoter. Grain from transgenic rice plants contained three times more iron than normal rice. To increase the iron content in the grain further, the researchers focused on iron transport within the plant (Lucca et al., 2002). Drakakaki et al. (2005) demonstrated endosperm-specific coexpression of recombinant soybean ferritin and *Aspergillus* phytase in maize, which resulted in significant increases in the levels of bioavailable iron. A similar result was achieved with lettuce (Goto et al., 2000).

Functional Metabolites

Unlike for vitamins and minerals, the primary evidence for the health-promoting roles of phytochemicals comes from epidemiological studies, and the exact chemical identities of many active compounds have yet to be determined. However, for select groups of phytochemicals, such as nonprovitamin A, carotenoids, glucosinolates, and phytoestrogens, the active compound or compounds have been identified and rigorously studied. A great irony of nature is that the body's natural metabolism involving oxygen also produces a host of toxic compounds called "free radicals." These compounds can harm body cells by altering molecules of protein and fat and by damaging DNA. Antioxidants counteract, or neutralize, the harmful effects of free radicals.

Epidemiologic studies have suggested a potential benefit of the carotenoid lycopene in reducing the risk of prostate cancer, particularly the more lethal forms of this cancer. Five studies support a 30% to 40% reduction in risk associated with high tomato or lycopene consumption in the processed form in conjunction with lipid consumption, although other studies with raw tomatoes were not conclusive (Giovinazzo et al., 2005). As a nonpolar carotenoid, lycopene is more soluble in a lipid base; in addition, carotenoid-binding proteins are broken down during processing, leading to greater bioavailability. While modifying polyamines to retard tomato ripening, Mehta et al. (2002) discovered an unanticipated enrichment in lycopene, with levels up by 2- to 3.5-fold compared with the conventional tomatoes. This is a substantial enrichment, exceeding that so far achieved by conventional means. This novel approach may work in other fruits and vegetables.

Stilbenes, including resveratrol (3,5,4'-trihydroxystilbene), are phenolic natural products that accumu-

late in a wide range of plant species, including pine (*Pinus* spp.), peanut (*Arachis hypogaea*), rhubarb (*Rheum* spp.), and grape (*Vitis vinifera*; Tropf et al., 1994). Resveratrol inhibits platelet aggregation and eicosanoid synthesis and is thought to contribute to improved heart function and lower blood cholesterol, based on epidemiological studies (Frankel et al., 1993; Pace-Asciak et al., 1995; Wieder et al., 2001). It was shown to have "chemopreventive" activity, preventing the formation of tumors in mouse skin bioassays, and, therefore, may help reduce cancer rates in humans (Jang et al., 1997). More recent studies appear to demonstrate that it mimics the life-extending effect on rodents of severe caloric restriction. This diet extends the life span of rodents by 30% to 50%, and even if it is started later it has a benefit proportionate to the remaining life span. The method of action is believed to be in protecting the sirtuins, genes implicated in DNA modification and life extension (Baur, 2006). The April 2008 purchase of Sirtris by Glaxo Smith Kline (Pollack, 2008) demonstrates that big pharma is now showing an interest in the arena of food functionality. Resveratrol glucoside production has been achieved in alfalfa (*Medicago sativa*), wheat, kiwi (*Actinidia deliciosa*), and tomato (Stark-Lorenzen et al., 1997; Hipskind and Paiva, 2000; Kobayashi et al., 2000; Szankowski et al., 2003; Niggeweg et al., 2004; Giovinazzo et al., 2005; Shin et al., 2006).

Other phytochemicals of interest include the flavonoids, such as tomatoes expressing chalcone isomerase, which show increased contents of the flavanols rutin and kaempferol glycoside; glucosinolates and their related products, such as indole-3 carbinol; catechin and catechol; isoflavones, such as genistein and daidzein; anthocyanins; and some phytoalexins (Table I). A comprehensive list of phytochemicals is outlined in Table II. Although there is a growing knowledge base indicating that elevated intakes of specific phytochemicals may reduce the risk of disease, such as certain cancers, cardiovascular diseases, and chronic degenerative diseases associated with aging, further research and epidemiological studies are still required to prove definitive relationships.

ANTINUTRIENTS, ALLERGENS, AND TOXINS

Plants produce many defense strategies to protect themselves from predators, and many of these, such as resveratrol and glucosinate, which are primarily pathogen-protective chemicals, also have demonstrated beneficial effects for human and animal health. Many, however, have the opposite effect. For example, phytate, a plant phosphate storage compound, is an antinutrient, as it strongly chelates iron, calcium, zinc, and other divalent mineral ions, making them unavailable for uptake. Nonruminant animals generally lack the phytase enzyme needed for digestion of phytate. Poultry and swine producers add processed phosphate to their feed rations to counter this. Excess phosphate is excreted into the environment, resulting

in water pollution. When low-phytate soybean meal is utilized along with low-phytate maize for animal feeds, the phosphate excretion in swine and poultry manure is halved. A number of groups have added heat- and acid-stable phytase from *Aspergillus fumigatus* to make the phosphate and liberated ions bioavailable in several crops (Lucca et al., 2002). To promote the reabsorption of iron, a gene for a metallothionein-like protein has also been engineered. Low-phytate maize was commercialized in the United States in 1999 (Wehrspann, 1998). Research indicates that the protein in low-phytate soybeans is also slightly more digestible than the protein in traditional soybeans. In a poultry feeding trial, better results were obtained using transgenic plant material than with the commercially produced phytase supplement (Keshavarz, 2003). Poultry grew well on the engineered alfalfa diet without any inorganic phosphorus supplement, which shows that plants can be tailored to increase the bioavailability of this essential mineral.

Other antinutrients that are being examined as possible targets for reduction are trypsin inhibitors, lectins, and several other heat-stable components found in soybeans and other crops. Likewise, strategies are being applied to reduce or limit food allergens (albumins, globulins, etc.), malabsorption and food intolerances (gluten), and toxins (glycoalkaloids, cyanogenic glucosides, phytohemagglutinins) in crop plants and undesirable aesthetics such as caffeine (Ogita et al., 2003). Examples include changing the levels of expression of the thioredoxin gene to reduce the intolerance effects of wheat and other cereals (Buchanan et al., 1997). Using RNA interference to silence the major allergen in soybean (p34, a member of the papain superfamily of Cys proteases) and rice (14- to 16-kD allergenic proteins by antisense; Tada et al., 1996), blood serum tests indicate that p34-specific IgE antibodies could not be detected after consumption of gene-silenced beans (Herman et al., 2003).

Biotechnology approaches can be employed to down-regulate or even eliminate the genes involved in the metabolic pathways for the production, accumulation, and/or activation of these toxins in plants. For example, the solanine content of potato has already been reduced substantially using an antisense approach, and efforts are under way to reduce the level of the other major potato glycoalkaloid, chaconine (McCue et al., 2003). Work has also been done to reduce cyanogenic glycosides in cassava through expression of the cassava enzyme hydroxynitrile lyase in roots (Siritunga and Sayre, 2003). When "disarming" plant natural defenses in this way, we need to be cognizant of potentially increased susceptibility to pests and diseases, so the base germplasm should have input traits to counter this.

IMPLICATIONS FOR SAFETY ASSESSMENT

On the surface, it may appear that the greater complexity involved in modifying the nutritional con-

tent of crop plants would necessitate more rigorous oversight than the simpler modifications. However, extensive research reported previously (International Life Sciences Institute, 2004a, 2004b) and updated in a more recent case study analysis (International Life Sciences Institute, 2008) indicates that existing oversight systems are more than adequate. Trait modifications with the addition of one or two genes that do not act on central or intermediary metabolism produce targeted, predictable outcomes, whereas major modifications of metabolic pathways can produce unanticipated effects. Therefore, it is very encouraging that the ever-evolving and increasingly sensitive and discriminating analytical technologies have been able to detect and assess the safety of these unanticipated effects. In addition, regulatory oversight of GM products has been designed to detect such unexpected outcomes.

At a very fundamental level, a recent report (Baack and Rieseberg, 2007) on genome-wide analyses of introgression from oak (*Quercus* spp.) to fruit flies indicates that a substantial fraction of genomes are malleable. Hybridization gives rapid genomic changes, chromosomal rearrangements, genome expansion, differential expression, and gene silencing (transposable elements). In the context of this sea of malleability, reports have demonstrated that GM crops have a composition more similar to the isogenic parental strain used in their development than to other breeding cultivars of the same genus and species and in some instances even the location in which they are grown, and on occasion the latter "terroir" effect demonstrated greater variation than breeding strategy. This effect has been observed at the proteome level for potato (Lehesranta et al., 2005), tomato (Corpillo et al., 2004), and wheat (Shewry, 2003). Parallel results have been observed at the metabolomic level for wheat (Baker et al., 2006) and potato (Catchpole et al., 2005). As more metabolic modifications are introduced, we must continue to study plant metabolism and the interconnected cellular networks of plant metabolic pathways to increase the likelihood of predicting pleiotropic effects that may occur as a result of the introduced genetic modification.

THE FUTURE OF CROP BIOTECHNOLOGY

Research to improve the nutritional quality of plants has historically been limited by a lack of basic knowledge of plant metabolism and the almost insurmountable challenge of resolving complex branches of thousands of metabolic pathways. With the tools now available to us through the fields of genomics and bioinformatics, we have the potential to fish in silico for genes of value across species, phyla, and kingdoms and subsequently to study the expression and interaction of transgenes on tens of thousands of endogenous genes simultaneously. With advances in proteomics, we should also be able to simultaneously quantify the levels and interactions of many proteins or follow posttranslational alterations that occur. With

these newly evolving tools, we are beginning to get a handle on the global effects of metabolic engineering on metabolites, enzyme activities, and fluxes. Right now, for essential macronutrients and micronutrients that are limiting in various regional diets, the strategies for improvement are clear and the concerns, such as pleiotropic effects and safe upper limits, are easily addressed. However, for many other health-promoting phytochemicals, clear links with health benefits remain to be demonstrated. Such links, if established, will make it possible to identify the precise compound or compounds to target and which crops to modify to achieve the greatest nutritional impact and health benefits. The achievement of this aim will be a truly interdisciplinary effort, requiring expertise and input from many disparate fields, ranging from the obvious human physiology and plant research to the less obvious "omics" and analytic fields.

With these emerging capabilities, the increase in our basic understanding of plant secondary metabolism during the coming decades will be unparalleled and will place plant researchers in the position of being able to modify the nutritional content of major and minor crops to improve many aspects of human and animal health and well-being.

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