IMPROVING THE NUTRIENT COMPOSITION OF PLANTS TO ENHANCE HUMAN NUTRITION AND HEALTH

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ABSTRACT
Plant foods contain almost all of the mineral and organic nutrients established as essential for human nutrition, as well as a number of unique organic phytochemicals that have been linked to the promotion of good health. Because the concentrations of many of these dietary constituents are often low in edible plant sources, research is under way to understand the physiological, biochemical, and molecular mechanisms that contribute to their transport, synthesis and accumulation in plants. This knowledge can be used to develop strategies with which to manipulate crop plants, and thereby improve their nutritional quality. Improvement strategies will differ between various nutrients, but generalizations can be made for mineral or organic nutrients. This review focuses on the plant nutritional physiology and biochemistry of two essential human nutrients, iron and vitamin E, to provide examples of the type of information that is needed, and the strategies that can be used, to improve the mineral or organic nutrient composition of plants.

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INTRODUCTION

The nutritional health and well-being of humans are entirely dependent on plant foods. Plants are critical components of the dietary food chain in that they provide almost all essential mineral and organic nutrients to humans either directly, or indirectly when plants are consumed by animals, which are then consumed by humans. Because plants are autotrophic, they can acquire elemental compounds and convert these into the building blocks (e.g. amino acids, fatty acids, nucleic acids, secondary metabolites) needed to make all complex macromolecules necessary to support plant growth and reproduction. Humans, on the other hand, require many of the same mineral nutrients as plants, but have additional requirements for various complex organic molecules. With the exception of vitamins B\textsubscript{12} and D, a plant-based food supply can ensure the adequate nutrition of humans at all stages of life.

Not all plant foods, however, contain all the essential nutrients needed for human health, nor do they usually contain given nutrients in sufficiently concentrated amounts to meet daily dietary requirements in a single serving. For instance, seed foods are good sources of carbohydrates, proteins, lipids, and lipid-soluble vitamins, but tend to have low concentrations of Fe and Ca. Leafy vegetables are good sources of most minerals and vitamins, but are less nutrient dense with respect to protein and carbohydrates. Fruits provide carbohydrates, water-soluble vitamins, and various carotenoids, but generally are minor
sources of protein and certain minerals. Thus, not only is a diverse, complex diet necessary to fully support human growth and health, but the concentration of various nutrients within the dietary mix also is an important determinant of whether nutritional requirements are being met.

Unfortunately, many people do not consume a sufficiently diverse diet. In the developing world, many low-income families exist on a simple diet composed primarily of staple foods (e.g. rice, wheat, maize) that are poor sources of some macronutrients and many micronutrients (22). As a result, recent estimates are that 250 million children are at risk for vitamin A deficiency (of which 250,000–500,000 will suffer irreversible blindness every year), 2 billion people (33% of the world’s population) are at risk for iron deficiency (infants, children, and women of reproductive age are particularly vulnerable), and 1.5 billion people are at risk for iodine deficiency (38). Furthermore, even in the United States, the average intake of fruits and vegetables was recently assessed to be only 3.4 servings per day, well below the five-per-day recommendation of the US National Research Council (110, 145). This recommendation is derived from numerous epidemiological studies, which suggest that the daily consumption of five or more servings of fruits and vegetables is associated with reduced risk for several types of cancer as well as other degenerative diseases (12). Many of these studies have implicated antioxidants, such as carotenoids (e.g. β-carotene and lycopene), tocopherols, ascorbic acid, and selenium, as contributors to the healthful properties of such a diet. Furthermore, fruits and vegetables also contain a vast and complex array of bioactive secondary compounds (nonessential for humans), collectively referred to as phytochemicals, that show promise in contributing to the promotion of good health and protection against human diseases (139, 165).

To ensure an adequate dietary intake of all essential nutrients and to increase the consumption of various health-promoting compounds, researchers have been interested in improving the nutritional quality of plants, with respect to both nutrient composition and concentration (13, 47, 161). In this article, we discuss the role that plant foods play in human nutrition and health, and review some of the efforts pertinent to plant nutrient composition. We then review our current knowledge regarding the physiological, biochemical, and molecular mechanisms that are involved in the eventual deposition of iron, a mineral nutrient, and vitamin E, an organic nutrient. Because it would be impossible to discuss all plant-derived nutrients in this article, we focus on just these two nutrients to provide examples of the type of information that is needed, and the strategies that can be used, to improve the mineral or organic nutrient composition of plants.
ROLE OF PLANTS IN HUMAN HEALTH

Essential Human Nutrients
Humans require an energy supply (in addition to water and oxygen) that can be provided by a mixture of carbohydrates, lipids, and protein (amino acids), as well as 17 mineral nutrients and 13 vitamins. Among the lipids, linoleic acid and linolenic acid cannot be synthesized by humans (65), and thus must be obtained from dietary sources (plants synthesize both of these fatty acids). Nine essential amino acids must be obtained in various amounts from ingested protein (90), which also provides dietary sulfur and nitrogen. The 15 remaining essential mineral nutrients and 13 vitamins are required in varying amounts, as indicated in Table 1. To provide examples of some of the higher daily nutrient requirements that would need to be met from dietary sources, Recommended Dietary Allowances (RDAs) are presented for adults (Table 1). RDAs are the daily levels of intake of essential nutrients judged to be adequate to meet the known nutrient needs of practically all healthy persons (110). RDAs vary with age, sex, and physiological status (i.e. pregnancy, lactation), and generally are lowest for newborn infants and highest for adult males and pregnant or lactating women.

Dietary standards have been around for over 100 years, and initially were developed as recommendations to alleviate starvation and associated nutrient-deficiency diseases during economic and wartime crises (57, 58). The US RDAs were first published in 1941, with the most recent, the 10th edition, published in 1989 (110). This soon will be replaced with a new set of values called the Dietary Reference Intakes (DRIs) (1) that are scheduled to be published in the year 2000. DRIs are being developed to encompass three existing values: RDAs, the Estimated Average Requirements, and the Tolerable Upper Intake Levels (66). The need for the new DRI values reflects the growing knowledge base regarding the roles of nutrients in human health. Vitamins, minerals, and other dietary constituents at varying levels are now known to be significant contributors to the reduction of risk of chronic diseases such as cancers, cardiovascular diseases, and degenerative diseases associated with aging (11, 20, 77, 139, 148, 165), in addition to alleviating the classical nutritional deficiency diseases. Thus, new guidelines are needed, especially with regard to upper limits of safe intake.

Plant foods can contribute significantly to human nutrition and health, because they contain almost all essential human nutrients. However, as noted in Table 1, nutrient composition varies among different plant foods, and nutrient content in a single serving rarely fulfills the RDA for any given vitamin or mineral. Note also that the content values are for whole, unprocessed and uncooked foods. Many vitamins and minerals are lost or greatly reduced in concentration during cooking, storage, or processing (127). Thus, enhancing the nutrient
concentration of many plant food products would contribute significantly to human nutrition and health.

Bioavailability

The total content, or absolute concentration, of a given nutrient in a food is not always a good indicator of its useful nutritional quality, because not all of the nutrients in food are absorbed. Human nutritionists use the term bioavailability to describe the proportion of an ingested nutrient that is digested, absorbed, and ultimately utilized (17). Digestion involves various physical, chemical, enzymatic, and secretory processes that combine to break down the food matrix as much as possible (keep in mind that cellulose cannot be digested because humans do not express a cellulase). These digestive processes serve to release and solubilize nutrients so they can diffuse out of the bulk food matrix to the enterocytes of the intestine. Absorption then depends on whether the nutrient is in an appropriate form (e.g. charged, complexed), whether the necessary absorptive transport systems are in place in the gut (this depends in part on the individual’s nutritional status), and whether inhibitory or promotive substances are present in the food matrix (90). Because of these issues, the bioavailability of many minerals and organic micronutrients varies greatly (110), and can be as low as 5% in the case of most plant sources of Fe (23) or in the case of Ca when it is present in foods as crystalline Ca-oxalate (40, 158). This raises the possibility that one strategy for improving plant nutritional quality could be merely to alter the composition of the food (e.g. changing the form of the stored nutrient, removing inhibitory compounds), in order to enhance the bioavailability of existing nutrient levels.

Phytochemicals

Besides the well-established minerals and vitamins, plants can provide an array of interesting, nonessential phytochemicals in the diet. Phytochemicals can be separated into several groups, based on their biosynthetic origin, with some groups containing several thousand chemically distinct compounds. These include flavonoids, flavones, phytosterols, phenols and polyphenols, phytoestrogens, glucosinolates, and indoles, to name but a few (11, 27, 33, 37, 69, 82, 152). With respect to human health, estimates of potential therapeutic value have been made for numerous compounds produced by plants. Unfortunately, most of our information in this area stems from epidemiological evidence that only can provide associations between health benefits and particular foods or classes of food components. Thus, the current research focus in this area is to identify the exact chemical(s) responsible for a given beneficial effect and to delineate the underlying biochemical and molecular mechanisms involved. For many groups of phytochemicals, we know little about their bioavailability, whether
Table 1: Essential human nutrients, daily requirements, and plant food sources

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Maximum adult RDA</th>
<th>Safe upper intake limits (relative to RDA)</th>
<th>Predominant plant source</th>
<th>Mean nutrient content (source)</th>
<th>Human health reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Minerals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>2000 mg</td>
<td>9X</td>
<td>Various</td>
<td>447 mg (kale)</td>
<td>94</td>
</tr>
<tr>
<td>Calcium</td>
<td>1200 mg</td>
<td>2X</td>
<td>Vegetables</td>
<td>48 mg (broccoli)</td>
<td>4</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1200 mg</td>
<td>2X</td>
<td>Various</td>
<td>376 mg (peanut)</td>
<td>4</td>
</tr>
<tr>
<td>Chloride</td>
<td>750 mg</td>
<td>5X</td>
<td>Various</td>
<td>60 mg (tomato)</td>
<td>102</td>
</tr>
<tr>
<td>Sodium</td>
<td>500 mg</td>
<td>5X</td>
<td>Various</td>
<td>79 mg (spinach)</td>
<td>102</td>
</tr>
<tr>
<td>Magnesium</td>
<td>350 mg</td>
<td>1X</td>
<td>Seeds, leafy vegetables</td>
<td>138 mg (wheat flour)</td>
<td>32</td>
</tr>
<tr>
<td>Iron</td>
<td>15 mg</td>
<td>5X</td>
<td>Seeds, leafy vegetables</td>
<td>1.47 mg (pea)</td>
<td>164</td>
</tr>
<tr>
<td>Zinc</td>
<td>15 mg</td>
<td>1X</td>
<td>Seeds</td>
<td>2.93 mg (wheat flour)</td>
<td>2</td>
</tr>
<tr>
<td>Manganese d</td>
<td>2–5 mg</td>
<td>1X</td>
<td>Seeds</td>
<td>4.9 mg (oat)</td>
<td>76</td>
</tr>
<tr>
<td>Fluoride d</td>
<td>1.5–4 mg</td>
<td>1X</td>
<td>Aerial tissues</td>
<td>0.11 mg (spinach)</td>
<td>79</td>
</tr>
<tr>
<td>Copper d</td>
<td>1.5–3 mg</td>
<td>1X</td>
<td>Seeds</td>
<td>1.14 mg (peanut)</td>
<td>91</td>
</tr>
<tr>
<td>Molybdenum d</td>
<td>75–250 µg</td>
<td>1X</td>
<td>Seeds</td>
<td>70 µg (oat)</td>
<td>119</td>
</tr>
<tr>
<td>Chromium d</td>
<td>50–200 µg</td>
<td>1X</td>
<td>Various</td>
<td>33 µg (potato)</td>
<td>105</td>
</tr>
<tr>
<td>Iodine</td>
<td>150 µg</td>
<td>13X</td>
<td>Various</td>
<td>12 µg (kale)</td>
<td>28</td>
</tr>
<tr>
<td>Selenium</td>
<td>70 µg</td>
<td>13X</td>
<td>Seeds</td>
<td>7.2 µg (peanut)</td>
<td>89</td>
</tr>
<tr>
<td><strong>Water-soluble vitamins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>60 mg</td>
<td>16X</td>
<td>Fruits, vegetables</td>
<td>42.2 mg (cantaloupe)</td>
<td>129</td>
</tr>
<tr>
<td>Niacin (vitamin B$_3$)</td>
<td>19 mg NE</td>
<td>150X</td>
<td>Seeds, leafy vegetables</td>
<td>9.9 mg NE (wheat flour)</td>
<td>68</td>
</tr>
<tr>
<td>Pantothenic acid d</td>
<td>4–7 mg</td>
<td>150X</td>
<td>Seeds</td>
<td>1.35 mg (oat)</td>
<td>146</td>
</tr>
</tbody>
</table>
### Vitamin B Complex

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
<th>Soluble</th>
<th>Source</th>
<th>Conversion Amount</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B₁ (Thiamin)</td>
<td>1.5 mg</td>
<td>Seeds</td>
<td>67X</td>
<td>0.64 mg (peanut)</td>
<td>121</td>
</tr>
<tr>
<td>Vitamin B₂ (Riboflavin)</td>
<td>1.7 mg</td>
<td>Cereal grains, leafy vegetables</td>
<td>125X</td>
<td>0.22 mg (wheat flour)</td>
<td>103</td>
</tr>
<tr>
<td>Vitamin B₃ (Folate)</td>
<td>200 µg</td>
<td>Seeds</td>
<td>50X</td>
<td>240 µg (peanut)</td>
<td>21</td>
</tr>
<tr>
<td>Vitamin B₆ (Pyridoxine)</td>
<td>2 mg</td>
<td>Cereal grains, leafy vegetables</td>
<td>0.35 mg (peanut)</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Vitamin B₉ (Biotin)</td>
<td>30–100 µg</td>
<td>Seeds</td>
<td>300X</td>
<td>13 µg (oat)</td>
<td>106</td>
</tr>
<tr>
<td>Vitamin B₁₂ (Cobalamin)</td>
<td>2.0 µg</td>
<td>Not found in plants</td>
<td>500X</td>
<td>0 (all plants)</td>
<td>60</td>
</tr>
</tbody>
</table>

### Fat-soluble vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
<th>Soluble</th>
<th>Source</th>
<th>Conversion Amount</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E</td>
<td>10 mg α-TE</td>
<td>Seeds, leafy vegetables</td>
<td>100X</td>
<td>1.23 mg α-TE (wheat flour)</td>
<td>154</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>1000 µg RE</td>
<td>Colored fruits and vegetables</td>
<td>5X (retinol)</td>
<td>2813 µg RE (carrot)</td>
<td>124</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>80 µg</td>
<td>Leafy vegetables</td>
<td>375X</td>
<td>350 µg (spinach)</td>
<td>155</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>10 µg</td>
<td>Not found in plants</td>
<td>4X</td>
<td>0 (all plants)</td>
<td>29</td>
</tr>
</tbody>
</table>

*Recommended dietary allowances (RDA) are the daily levels of intake of essential nutrients judged to be adequate to meet the known nutrient needs of practically all healthy persons. Values presented are the highest RDA either for male or female adults, excluding pregnant or lactating women. Values are from Reference 110.

*The concept assumes that there is individual variation in both requirement for the nutrient and tolerance for high intake. The safe upper intake limit is associated with a low probability of adverse side effect. The authors are not advocating intake of supplements at these levels. Values were derived from References 83, 110, and 168.

*Concentrations are for 100 g raw, edible portion of food; values are representative for predominant source and are not the highest known example. All seed sources represent whole-seed or whole-grain values. Note that water content varies among sources. Values are from References 138 and 154a.

*Recommended values for these nutrients are provided as estimated safe and adequate daily dietary intake ranges, because less information is available on which to base the RDAs. Values are from Reference 110.

*One mg NE (niacin equivalent) is equal to 1 mg of niacin or 60 mg of dietary tryptophan.

*One mg α-TE (α-tocopherol equivalent) is equal to 1 mg (R,R,R)-α-tocopherol.

*Preformed vitamin A is not found in plant foods. However, plants contain a number of provitamin A carotenoids (e.g., β-carotene), which can be metabolized to vitamin A.

*Vitamin A activity is expressed in retinol equivalents (RE). One mg RE is equal to 1 µg all-trans retinol, 6 µg all-trans β-carotene, or 12 µg of other provitamin A carotenoids.
the absorbed compounds are metabolized into active or inactive forms in the body, or their modes of action at the cellular, biochemical, or molecular level.

PLANT IMPROVEMENT THROUGH CULTIVAR SELECTION

The primary objective of modern agriculture and breeding programs over the past 50 years has been to increase productivity by increasing yields. This has been achieved primarily by selecting for resistance to diseases, increased fruit set and size, specific plant growth forms, increased grain fill, etc. The quest for increased yield is without a doubt a primary concern for a growing world population, and should be continued with vigor. However, equally as important, but often overlooked, are the nutrient composition and density of crops, especially with regard to micronutrients.

When nutrient content has been assessed in various plants, significant genotypic variation has been observed for minerals, vitamins and phytochemicals. For example, pod Ca concentration varied nearly twofold among snap bean genotypes (118), β-carotene concentrations varied fourfold among broccoli cultivars (132), and folate concentrations varied fourfold among red beet cultivars (157). These few examples highlight the fact that significant variation in nutrient content already exists within the germplasm of these species, and probably exists in many others. Thus, classical breeding approaches can and are being used to provide nutritionally improved cultivars (135, 151). In addition, genotypes with dissimilar nutrient concentration are helping to identify the genetic (144) or physiological (54) basis for nutrient variation. Clearly, more advances will be possible if attention is given to the evaluation of currently available germplasm resources (149).

IMPROVEMENT STRATEGIES FOR MINERAL NUTRIENTS: IRON AS AN EXAMPLE

General Considerations

Minerals can be grouped into three categories: the macronutrient minerals (N, S, P, Ca, K, Mg) that are needed in highest concentration by plants (mg/g dry weight range), the micronutrient minerals (Fe, Mn, B, Cl, Zn, Cu, Mo, Ni) that are needed in lesser amounts (µg/g dry weight range), and various generally nonessential minerals (e.g. Na, F, Se, Cr, I) that are found in plant tissues in varying concentrations (71). A plant’s ability to increase the total content of a mineral from any one of these categories always depends on soil composition and the availability of that mineral in the plant’s environment. Different improvement strategies may be needed for essential and nonessential minerals, depending on the existence of specific or nonspecific transport systems
and the capacity for safe bioavailable storage in the edible tissues. Additionally, because energy costs to the plant may dictate that content changes are more feasible at the \( \mu g \) level than the mg level, percentage changes in mineral content may be more dramatic for the micronutrient and nonessential minerals.

The mineral composition of each plant organ is determined by a sequence of events that begins with membrane transport in the roots, proceeds to the xylem system for transit to the vegetative organs, may involve temporary storage in stem or leafy tissues, in some cases utilizes mobilization via the phloem pathway, and concludes with deposition in one or more cellular compartments (see Figure 1). All of these processes are integrated and regulated by the plant to

![Figure 1](image-url)

*Figure 1* Whole plant schematic of the processes relevant to Fe transport and accumulation in higher plant tissues. Potential control points that would influence the movement of Fe from one compartment to the next include: (A) Fe acquisition/uptake phenomena, including the release of compounds by roots to chelate or solubilize soil Fe; (B) intracellular/intercellular transport, including the involvement of xylem parenchyma; (C) transpiration rates of vegetative tissues; (D) storage and remobilization phenomena; (E) Fe-chelate expression and capacity for phloem Fe loading; (F) phloem transport capacity of photoassimilates from a given source region; (G) communication of shoot Fe status via phloem-mobile signal molecules to regulate root processes.
ensure that adequate, but not toxic, quantities of mineral nutrients are available for the plant’s growth and development. For any given mineral, a holistic understanding of the relevant transport and partitioning mechanisms, and the molecular to whole-plant factors that regulate them, is critical to enable improvement strategies to be developed. Strategically, one must determine the process or processes that rate limit the eventual deposition of each mineral in a given structure, such that these can be targeted for genetic or molecular modification. Our current understanding of these processes in iron nutrition is given as an example.

Iron Processes in Plants

Iron is an essential nutrient found in leaves and other plant tissues, typically at low concentrations (<200 µg/g dry weight). As a component of various redox and iron-sulfur enzymes (100), iron plays an important role in general plant metabolism. Additionally, it is essential for chlorophyll formation (117). However, iron is an extremely reactive transition metal that can catalyze the formation of free radical species (59). Because these secondary species can damage lipids, proteins, or nucleic acids (77), Fe uptake is tightly regulated, and most of the Fe that does enter the plant must be chelated or sequestered in nonreactive forms. Thus, to improve plant Fe composition, not only do acquisition processes need to be upregulated, but attention must also be paid to how any excess Fe is handled as it moves through or is sequestered within the plant.

ROOT IRON ACQUISITION

Higher plants utilize one of two strategies for Fe acquisition (101). Strategy I involves an obligatory reduction of ferric iron (usually as an Fe[III]-compound) prior to membrane influx of Fe²⁺; this strategy is used by all dicotyledonous plants and the non-grass monocots. Strategy II (used by grasses) employs ferric chelators, called phytosiderophores, that are released by roots and chelate ferric iron in the rhizosphere. The Fe(III)-phytosiderophore is absorbed intact via a plasmalemma transport protein. When plants of either strategy are challenged with Fe-deficiency stress, the processes associated with one or the other strategy are upregulated in the plant’s root system.

Mechanistically, Strategy I plants utilize a plasmalemma reductase that transfers an electron from an internal reductant to an external Fe(III)-chelate (63). Reduced Fe²⁺ is released from the chelate and is transported across the root-cell plasmalemma via a ferrous transport protein (34, 39). At present, root Fe(III) reductase activity has been characterized in a number of species (107), but no plant Fe(III) reductase has been isolated and sequenced. However, four candidate genes (frohA, frohB, frohC, and frohD), suggested to encode Fe(III) reductases, have been identified in Arabidopsis by PCR, using degenerate primers based on conserved motifs found in yeast Fe(III) reductases (122, 123).
More information is known about the Fe$^{2+}$ transporter. The *Arabidopsis* *IRT1* gene (for Iron Regulated Transporter) was identified by functional complementation of a yeast mutant defective in iron uptake (34). Expression of *IRT1* is localized to roots in *Arabidopsis*, and is induced when plants are challenged with Fe deficiency.

Strategy II plants release one of a group of closely related phytosiderophores belonging to the mugineic acid family (147). Phytosiderophores are low-molecular-weight peptides derived from methionine (109, 134); their biosynthetic pathway is fairly well established (97, 108). A few of the critical biosynthetic enzymes have recently been identified (62, 73, 74), as a result of differences observed between Fe-sufficient and Fe-deficient plants. Strategy II plants require at least two membrane transport systems for phytosiderophores: one to facilitate the efflux of uncomplexed phytosiderophore and the other the influx of Fe(III)-phytosiderophore. Release follows a diurnal periodicity in most species (167) and may involve vesicular targeting to the plasmalemma (112). Fe(III)-phytosiderophore influx is generally increased in Fe-deficient plants and the uptake system appears capable of transporting most of the identified Fe(III)-phytosiderophore species (96). Both high-affinity and low-affinity phytosiderophore uptake systems have been shown to function in maize (156). Neither of the transporters has been identified at the protein or gene level, but a transport-defective maize mutant (*ys1*) may prove useful in this regard (156).

Although much has been learned about the mechanisms of iron acquisition in higher plants, including the upregulation of root biosynthetic and transport systems in response to Fe deficiency, we only have limited understanding of the molecular regulation of these processes. Recent evidence suggests that the control may lie in the shoot tissues. Studies with two Fe-hyperaccumulating pea mutants demonstrated that shoot-to-root transmission of a phloem-mobile signal was responsible for the elevated rates of root Fe(III) reduction (53). Root reductase activity in wild-type, Fe-sufficient pea also was shown to be dynamically modulated throughout the plant’s life cycle in response to whole-plant Fe demand (49). These and other studies (84, 125) indicate that whole-plant Fe status is somehow monitored and assessed by the shoot tissues, with Fe need or Fe demand subsequently communicated to the roots, probably through an intracellular network (93). Because Fe(III) reduction is thought to be the rate-limiting Fe acquisition process in Strategy I plants (55, 166) and the entire phytosiderophore synthesis and transport cascade is critical for Strategy II plants (25, 156), it is no wonder that these processes are tightly regulated by the shoot to prevent toxic accumulation of Fe. The phloem-mobile signal compound has yet to be identified, but possible candidates include hormones, Fe-binding compounds, and even re-translocated Fe (10, 85, 98, 125, 141). Identification of the signal and a characterization of its molecular interaction
with Fe-status-responsive genes (e.g. *IRT1*) is crucial if we wish to control/enhance root acquisition of Fe.

**TRANSPORT TO AND STORAGE IN EDIBLE ORGANS** Once absorbed by root epidermal or cortical cells, Fe is transported radially to the root cortex for loading into the xylem pathway. It has been suggested that the Fe(II)-chelating peptide, nicotianamine (141), may act to stabilize free Fe$^{2+}$ and assist in its intracellular trafficking to the xylem parenchyma (81, 131, 160). However, because Fe moves within the xylem pathway as Fe(III)-citrate (100, 163), the Fe$^{2+}$ absorbed by Strategy I plants would appear to be oxidized to Fe$^{3+}$ at some location within the root, presumably in a controlled manner. Oxidation and subsequent citrate chelation may occur at the site of Fe uptake, because the nicotianamine-less tomato mutant, *chloronerva*, is able to transport Fe to its shoots (115). In Strategy II plants, symplasmic citrate chelation could similarly occur in the root periphery where Fe is absorbed as the ferric form. The processes of Fe movement through roots and within the xylem system may not be rate-limiting, because mutants that exhibit elevated Fe uptake capacity demonstrate excessive Fe hyperaccumulation in leaves (56, 80).

Transport in the xylem pathway carries Fe to all transpiring organs and delivers it initially to the organ’s apoplastic compartment (100). A combination of redox reactions, pH equilibria, and transport processes will determine the eventual fate of Fe within the tissue, and thereby influence its bioavailability as a human food source. Fe(III)-citrate can be reduced by light energy (18), a plasmalemma-localized reductase (19), or possibly apoplastic ascorbate (95) to generate free Fe$^{2+}$, which can be absorbed by leaf cells through an Fe$^{2+}$ transport protein (19). Alternatively, the Fe$^{2+}$ can reoxidize and precipitate in the cell wall space, possibly as Fe-hydroxide or Fe-phosphate species. Fe$^{2+}$ absorbed by leaf cells can be used in various enzymes, assist in chlorophyll biosynthesis, or be stored within the chloroplastic iron storage protein, phytoferritin (14, 15, 116). How Fe is handled within the symplasm and transported between cytoplasm and various organelles is poorly understood, although nicotianamine may play a role (130). An Fe-citrate transporter may aid the transport of Fe into chloroplasts (87), where ascorbate is thought to facilitate Fe partitioning into phytoferritin (86). Iron can be stored in phytoferritin for future use; phytoferritin also can be used to sequester excess Fe (92).

Some of the shoot Fe is exported to developing sinks and growing roots via the phloem pathway. Studies with the *brz* and *dgl* Fe-hyperaccumulating pea mutants demonstrated that Fe must be chelated prior to phloem loading (48), and that elevated rates of Fe loading are possible (99), presumably due to the overexpression of a chelator. In normal plants, Fe mobilization to developing
seeds is rate-limited by the level of synthesis of the phloem-mobile chelator (48); this possibly serves to prevent Fe overload in the reproductive propagules. Little is known about the nature of the chelator, the compartment in which Fe chelation occurs, or the mechanism of phloem Fe loading (52, 140, 159).

**Strategies for Iron Improvement**

Plant sources of Fe include both xylem-fed leafy vegetables and phloem-fed seeds. Increasing the Fe content of either type usually necessitates increases in total Fe input to the plant, and may require modifications to whole-plant partitioning. Unfortunately, the homeostatic processes that control Fe influx and movement throughout the plant (see Figure 1) appear tightly matched to minimize Fe toxicity at all points within the system (52). Thus, if one step is altered to allow a higher flux of Fe, the next step may not necessarily conduct this enhanced flux. For instance, when two Fe(III) reductase genes from yeast (FRE1 and FRE2) were constitutively expressed in tobacco (128), total root reductase activity was enhanced fourfold relative to Fe-grown controls, but leaf Fe content was increased only 50% in the transformed plants. This suggests that the activity and/or spatial localization of the Fe\(^{2+}\) uptake system was not enhanced significantly to take advantage of the excess Fe\(^{2+}\) generated by the yeast reductases. More significant improvements in total Fe uptake may require a holistic approach involving overexpression of multiple components of the Fe acquisition system, overexpression of the Fe-status signal molecule, or identification and expression of transcriptional regulators that mediate iron deficiency responses, in order to activate all necessary root processes (9, 53).

In cases of plant organs, such as seeds, whose predominant supply of nutrients is provided via the phloem pathway, improvements in Fe content will require modifications to the phloem loading system. Overexpression of a phloem-mobile Fe-chelator can enable increased phloem Fe transport (99), but in species like pea, in which 75% of the shoot Fe content already is localized to the seeds (49), an increased uptake and delivery of Fe to the loading regions will also be required, preferably to coincide with the period of seed fill to prevent toxic accumulation in the leaves. Alternatively, in cereals such as rice, whose seeds import only about 4% of total shoot Fe (MA Grusak, unpublished), targeting increased phloem-mobile chelate expression to source regions that contain available Fe could help to increase seed Fe content. The Fe transported to seeds must then be sequestered in a nonreactive form, although phytoferritin expression may increase automatically in response to the elevated Fe load (92). Modifying seeds to store the excess Fe in heme-containing enzymes, or chelated to peptides, might further enhance the seed’s Fe nutritional quality by improving bioavailability (7, 45).
IMPROVEMENT STRATEGIES FOR ORGANIC NUTRIENTS: VITAMIN E AS AN EXAMPLE

General Considerations
Plants contain and elaborate many unique, interconnected biochemical pathways that produce an astonishing array of organic compounds that not only perform vital functions in plant cells, but also are essential or beneficial for human nutrition. One such class of compounds is the tocopherols (collectively known as vitamin E), a class of lipid soluble antioxidants that is synthesized only by plants and other photosynthetic organisms. The essential nutritional value of tocopherols was recognized more than 70 years ago and the compound responsible for the highest vitamin E activity was first identified specifically as α-tocopherol in 1922 (36). Despite the well-documented benefits of tocopherols in human diets (20, 148), only recently has significant progress been made at the molecular level regarding the synthesis and accumulation of tocopherols in plant tissues.

The low levels of activity and the membrane-bound nature of plant tocopherol biosynthetic enzymes have historically made the isolation of the corresponding genes, via protein purification, a daunting task. In this regard, tocopherols and other important lipid- and water-soluble plant-derived organic nutrients (e.g. carotenoids, folate, biotin, thiamin) have much in common. While the classical biochemical approaches of purification, enzyme assays, and radiolabeled tracer studies have been and will continue to be invaluable in furthering our understanding of the biosynthesis and transport of organic nutritional components, these approaches are limited in many ways. Often, protein stability and enzyme activity levels are low, substrates for biochemical assays are often not commercially available, and multiple components of a single biosynthetic step cannot be biochemically resolved.

Biochemical approaches can be complemented by genetic and molecular approaches in which one uses the organism to identify which steps are important in a biochemical pathway. Over the past several years, new genomic technologies, combined with the increasing ease of integrating molecular, genetic, and biochemical approaches in the field of plant biochemistry (16, 126, 153), have allowed researchers to make significant progress in dissecting the biosynthetic pathways for several classes of organic plant nutrients. The most successful in this regard has been the carotenoid pathway, in which almost all the biosynthetic enzymes have been cloned during the past several years (reviewed in 26). More recently, enzymes involved in folate and thiamin synthesis (8, 111, 120), biotin synthesis (5), and tocopherol synthesis (44, 78, 113, 114, 133a) have also been cloned. These genes undoubtedly will be used in the future to study and manipulate the synthesis of these nutrients at the molecular and biochemical levels,
with the ultimate goal of modifying the levels of these nutrients in agronomically important plants. The remainder of this section focuses on recent progress in the molecular dissection and manipulation of the tocopherol biosynthetic pathway in plants. \( \alpha \)-Tocopherol manipulation is presented here as a specific example of the potential of such integrative approaches for manipulation of plant-derived phytochemicals.

**Structures and Functions of Tocopherols**

The general structures of tocopherols are shown in Figure 2. All tocopherols are amphipathic molecules in which the hydrophobic tail associates with membrane lipids and the polar head groups remain at the membrane surface. \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \)-tocopherols differ only in the number and position of methyl substituents on the aromatic ring, with \( \alpha \)- having three, \( \beta \) and \( \gamma \)- having two, and \( \delta \)-tocopherol having only one substituent. Tocotrienols also occur in plants, differing from the tocopherols only in the degree of saturation of their hydrophobic tails. Tocopherols have saturated side chains and are the most common in plants. Tocotrienols are thought to be either biosynthetic intermediates that accumulate when specific steps in tail synthesis are blocked, or end products that specifically accumulate in some tissues, often to high levels (136).

The best-characterized and arguably most important function of tocopherols in biological membranes is to act as recyclable chain-reaction terminators of polyunsaturated fatty acid (PUFA) free radicals generated by lipid oxidation (41, 72). The in vivo antioxidant activities of tocopherols against lipid oxidation are \( \alpha > \beta \equiv \gamma > \delta \) with one molecule of each tocopherol protecting up to 220, 120, 100, and 30 molecules of PUFA, respectively, before being consumed (42). However, the relative antioxidant activity of tocopherols when tested in fats and oils in vitro is reversed to \( \delta > \beta \equiv \gamma > \alpha \).
Of the different tocopherol species present in foods, α-tocopherol is the most important to human health and has the highest vitamin E activity (100, 50, 10, and 3 percent relative activity for α-, β-, γ- and δ-tocopherols, respectively) (72). Naturally synthesized α-tocopherol occurs as a single (R,R,R)-α-tocopherol isomer. Chemically synthesized α-tocopherol, the most common tocopherol in vitamin E supplements, is a racemic mixture of eight different stereoisomers that range from 21% to 100% activity, relative to (R,R,R)-α-tocopherol (35). Although α-, β-, γ- and δ-tocopherols are absorbed equally during digestion, (R,R,R)-α-tocopherol is preferentially retained and distributed throughout the body (154). This retention is mediated by a hepatic tocopherol binding protein that shows a marked preference for α-tocopherol over β-, γ- and δ-tocopherols and non-(R,R,R)-α-tocopherol species (64). Over the past 20 years, a large and convincing body of epidemiological evidence has indicated that vitamin E supplementation at therapeutic doses (400 International Units, or approximately 250 mg of [R,R,R]-α-tocopherol daily) results in decreased risk for cardiovascular disease and cancer, aids in immune function, and prevents or slows a number of degenerative disease processes in humans (20, 148, 154). Note that this intake is much higher than the current adult RDAs for α-tocopherol (8 mg adult women, 10 mg adult men), levels intended merely to prevent a deficiency of this vitamin.

**Plant Oils: The Major Dietary Source of Tocopherols**

Plant tissues vary enormously in their total tocopherol content and tocopherol composition (Table 2), with concentrations ranging from extremely low levels in potato to very high levels in oil palm leaves and oil seeds (104). In green leafy tissues, α-tocopherol is often the most abundant tocopherol; however, such tissues contain relatively low concentrations of total tocopherols (i.e. between 10 and 50 µg/g fresh weight) (61, 104). This predominance of α-tocopherol in photosynthetic tissues presumably reflects a critical and highly conserved structural or functional role.

Unlike photosynthetic tissues, seeds often are more concentrated in total tocopherols, with their corresponding oils generally containing from 500 to 2000 µg/g tocopherols (61, 104). However, in most seed crops, including those from which the major edible oils are derived, α-tocopherol is present only as a minor component (150; Table 2). Nonetheless, seed oils still represent the major source of naturally derived dietary α-tocopherol due to the large amount of vegetable oils in the average American diet.

**The Tocopherol Biosynthetic Pathway**

The biosynthetic pathway for tocopherol synthesis in higher plants and algae (Figure 3) was elucidated in the early 1970s from precursor/product studies.
using radiolabeled intermediates. Through these efforts it was shown that photosynthetic organisms synthesize α-tocopherol using a common set of enzymatic reactions (162). The first step in the pathway is the formation of homogentisic acid (HGA), the aromatic precursor common to both tocopherols and plastoquinones (162), by the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPDase). The HPPDase enzyme locus (PDS1) has been identified by mutant analysis in Arabidopsis and shown to be essential for tocopherol and plastoquinone biosynthesis in plants (113). Several laboratories have now isolated cDNAs encoding HPPDase from various plant species and, surprisingly, have shown it to be a cytosolic enzyme (44, 114). HPPDase represents the first enzyme of the tocopherol biosynthetic pathway to be cloned from any photosynthetic organism.

HGA is subject to phytlation or prenylation (phytyl-PP and solanyl-PP, C$_{20}$ and C$_{45}$, respectively) to form the first true tocopherol and plastoquinone intermediates, 2-methyl-6-phytylplastoquinol and 2-methyl-6-solanylplastoquinol-9, respectively. Genetic studies have identified a single locus in Arabidopsis (the PDS2 locus) whose mutation disrupts both phytlation and prenylation, suggesting both reactions are mediated by a single enzyme (113). This activity is
Figure 3  Tocopherol biosynthetic pathway. The pathway shown is present in all photosynthetic organisms. Enzymatic activities are labeled in black boxes. The sequence of steps to α-tocopherol after addition of the phytol tail to HGA is the most widely accepted of many possible sequences proposed from biochemical studies. HPPDase is generally accepted as having a cytosolic localization; all other enzymes are presumably localized to plastids.
the branch point for the tocopherol and plastoquinone biosynthetic branches of the pathway, and represents a second potentially key enzymatic step regulating flux through the pathway. In addition to the level and activity of the enzyme encoded by the \textit{PDS2} locus, the availability of various substrates for the reaction (HGA, solanyl-PP, GGDP, and phytol-PP) also are potentially important in determining the total amount and ratios of tocopherol, tocotrienol, or PQ intermediates made in a tissue. For tocopherols, production of the hydrophobic tail would minimally require a (possibly specific) GGDP synthase and a reductase for saturating GGDP to generate phytol-PP. A GGDP reductase has recently been cloned that is active toward both free GGDP and geranylated chlorophyll derivatives (78). In the coming years, overexpression of this enzyme should allow determination of its involvement in tocopherol synthesis.

2-Methyl-6-phytylplastoquinol is the common intermediate in the synthesis of all tocopherols. The next steps in \textit{\alpha}-tocopherol synthesis are ring methylations and ring cyclization. The preferred reaction sequence for \textit{\alpha}-tocopherol synthesis in isolated spinach chloroplasts is thought to be: (a) ring methylation at position 3 to yield 2,3-dimethyl-6-phytylplastoquinol, (b) cyclization to yield \textit{d-7,8 dimethyltocol} (\textit{\gamma}-tocopherol), and finally (c) a second ring methylation at position 5 to yield \textit{\alpha}-tocopherol (137). The first ring methylation reaction is common to both tocopherol and plastoquinone synthesis and is thought to be carried out by a single enzyme that is specific for the site of methylation on the ring but has broad substrate specificity and accommodates both classes of compounds (24, 137). The second ring methylation enzyme (\textit{\gamma}-tocopherol methyltransferase) has an enzymatic activity distinct from the first and has been purified from both higher plants and algae (31, 67, 133). The tocopherol cyclization enzyme has been purified to homogeneity and biochemically characterized from \textit{Anabena variabilis} (142, 143).

Although \textit{\alpha}- and \textit{\gamma}-tocopherol biosynthesis proceeds as described above, it is not entirely clear how \textit{\delta}- and \textit{\beta}-tocopherols are produced. Although not commonly found in all plant tissues, \textit{\delta}- and \textit{\beta}-tocopherols can nonetheless be present at relatively high levels in certain tissues, most notably seeds. It is most likely that \textit{\delta}- and \textit{\beta}-tocopherols are synthesized by a subset of the same complement of enzymes involved in \textit{\alpha}-tocopherol synthesis and only accumulate under conditions when one or both methylation enzymes are rate limiting. The overall tocopherol composition is therefore determined by the combined activities and substrate specificities of the tocopherol cyclase and two methylation enzymes present in a given tissue.

**Strategies and Gene Targets for \textit{\alpha}-Tocopherol Improvement**

The tocopherol biosynthetic enzymes described above can be classified into two general groups: those predominantly affecting quantitative aspects of the
pathway (flux through the pathway) and those predominantly affecting qualitative aspects of the pathway (the relative amounts of \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \)-tocopherols produced). Current evidence suggests that steps involved in the formation and phytylation of HGA (HPPDase and the prenyl/phytyl transferase) and production of the phytol tail (GGDP synthase and GGDP reductase) function in a quantitative manner to regulate flux through the pathway (43). The subsequent cyclization and methylation reactions function primarily in a qualitative manner to regulate the tocopherol composition of a given plant tissue (30).

Quantitative manipulation of tocopherol levels requires metabolic engineering of what are likely to be multiple enzymatic activities in order to increase carbon flux through the pathway. HPPDase and GGDP reductase have already been cloned, and as other relevant pathway enzymes are cloned, quantitative manipulation of tocopherol levels in plant tissues may indeed become a reality. Qualitative manipulation of the existing tocopherol composition in a tissue would require positive or negative alteration of one or both of the methyltransferases. Negative alteration would cause accumulation of specific biosynthetic intermediates (\( \beta \)-, \( \gamma \)-, and \( \delta \)-tocopherols), while positive manipulation would result in the conversion of any biosynthetic intermediates to the pathway end product, \( \alpha \)-tocopherol. A tocopherol biosynthetic enzyme that is likely to have the greatest impact on the levels of \( \alpha \)-tocopherol accumulated in a tissue is the final enzyme of the pathway, \( \gamma \)-tocopherol methyltransferase.

IMPROVING DIETARY \( \alpha \)-TOCOPHEROL LEVEL BY MANIPULATING \( \gamma \)-TOCOPHEROL METHYLMTRANSFERASE (\( \gamma \)-TMT) ACTIVITY Although the most highly consumed vegetable oils in American diets (i.e. soybean, corn, and rapeseed oils) (3) contain very high levels of total tocopherol, these oils are relatively poor sources of \( \alpha \)-tocopherol (the form with the highest vitamin E activity), because \( \gamma \)-tocopherol predominates. As described earlier, \( \gamma \)-tocopherol is methylated to form \( \alpha \)-tocopherol in a reaction catalyzed by the enzyme \( \gamma \)-tocopherol methyltransferase (\( \gamma \)-TMT). These observations suggest that \( \gamma \)-TMT activity is likely limiting in the seeds of most agriculturally important oil crops and may be responsible for the low proportion of \( \alpha \)-tocopherol synthesized and accumulated. As such, \( \gamma \)-TMT is a prime molecular target for manipulation of \( \alpha \)-tocopherol levels in crops.

Given the levels and types of tocopherols in most of the important oil seed crops (Table 2), the following hypothetical scenario is an example of the impact that altering \( \gamma \)-TMT activity in seeds could have on the average daily intake of vitamin E. It has been estimated that between 20% and 25% of the calories consumed in American diets are derived from seed oils, with soybean oil accounting for \( \sim \)70% of the edible oil consumed (28–38 g daily). However, though 1 g of soybean oil contains 1.2 mg total tocopherols, only 7% is \( \alpha \)-tocopherol (6). One would need to consume 190–380 g of soybean oil daily
Nutritional improvement of plants (1800–3600 calories) in order to obtain the recently recommended daily intake of 15–30 mg α-tocopherol (154). If all the γ-tocopherol in soybean oil could be converted to α-tocopherol by overexpressing γ-TMT activity in seeds, 28–38 g of such an oil would provide 26–36 mg of α-tocopherol per day, a >tenfold increase over existing soybean oil. Note that this could be achieved without needing to alter the level of total tocopherols in the oil.

Isolation of genes encoding γ-TMT from Synechocystis PCC6803 and Arabidopsis

In order to isolate a cDNA encoding γ-TMT, complementary molecular genetic approaches were pursued concurrently in Arabidopsis and the photosynthetic bacteria Synechocystis PCC6803 (133a). These two model organisms were selected because both synthesize α-tocopherol of identical stereochemistry by presumably identical pathways (162), and both are highly tractable genetic, molecular, and biochemical systems. The ease with which gene disruption (46) can be used to test gene function in Synechocystis, combined with the recent report of the complete Synechocystis genome sequence (75), provided a unique opportunity for taking a genomics-based approach toward identifying genes encoding tocopherol biosynthetic enzymes.

By searching the Synechocystis genomic database with the Arabidopsis HPP-Dase protein sequence, a single open reading frame (ORF) with high homology was identified. The Synechocystis HPPDase gene was located within a 10-ORF operon. Because bacteria often organize enzymes of biosynthetic pathways into operons to ensure their coordinate regulation, it was hypothesized that the 10-ORF operon might also contain one or more genes that encode for additional enzymes involved in tocopherol synthesis in Synechocystis.

Examination of this operon identified one ORF (SLR0089) that shared a high degree of similarity to Δ-(24)-sterol-C-methyltransferases. SLR0089 also contained a predicted leader peptide that would target the protein to the bacterial plasma membrane, the site of tocopherol synthesis in this organism. To test the hypothesis that SLR0089 encodes a Synechocystis tocopherol methyltransferase, gene replacement experiments were performed to create a SLR0089 null mutant that could be analyzed for alterations in the normal Synechocystis tocopherol profile. Wild-type Synechocystis synthesizes greater than 95% of its total tocopherols as α-tocopherol (Table 2). The SLR0089 null mutant was unable to synthesize α-tocopherol and instead accumulated the immediate precursor γ-tocopherol as its sole tocopherol, a phenotype consistent with a disruption of γ-TMT activity. Expression of the SLR0089 open reading frame in Escherichia coli, followed by activity assays, directly demonstrated that the expressed enzyme was able to convert γ-tocopherol to α-tocopherol in vitro.

Having conclusively defined SLR0089 as a γ-TMT, its protein sequence was used to identify an ortholog from the Arabidopsis database. The Arabidopsis protein also demonstrated γ-TMT activity when expressed in E. coli (133a).
OVEREXPRESSION OF \(\gamma\)-TMT IN ARABIDOPSIS SEEDS INCREASES \(\alpha\)-TOCOPHEROL CONTENT

Having cloned a higher plant \(\gamma\)-TMT, the hypothesis could be tested that \(\gamma\)-TMT is a key and limiting enzyme regulating the \(\alpha\)-tocopherol composition of seeds. For this experiment, *Arabidopsis* was chosen as the model system because its seeds are composed of >95% \(\gamma\)-tocopherol and \(~\)1% \(\alpha\)-tocopherol. In such a system, even small increases in \(\alpha\)-tocopherol levels could be easily detected. The *Arabidopsis* \(\gamma\)-TMT cDNA was overexpressed on a seed specific promoter; pooled segregating T2 seeds from primary transformants were analyzed for changes in tocopherol content and composition. Several independent \(\gamma\)-TMT overexpressing lines contained 85–95% of their total tocopherol pool as \(\alpha\)-tocopherol (133a), a \(^{>\text{80-fold}}\) increase over wild-type controls in \(\alpha\)-tocopherol levels. Importantly, total seed tocopherol levels were not altered in these plants. Given the differing vitamin E potency of \(\alpha\)-, \(\beta\)-, \(\gamma\)- and \(\delta\)-tocopherols, the total vitamin E activity of 50 g of wild-type *Arabidopsis* seed oil would be 7.5 IU, while that of transgenic lines would be 67.5 IU, a ninefold increase in total vitamin E activity of the oil without increasing total tocopherols! This represents the first example of increasing a vitamin level in a plant tissue by molecular manipulation in plants. Similar increases in vitamin E activity as a result of \(\gamma\)-TMT overexpression can be envisioned for commercially important oils.

ISSUES AND PROSPECTS

Thomas Jefferson wrote: “The greatest service which can be rendered any country is, to add an useful plant to its culture” (70). Clearly, the development of nutritionally improved crops has immense significance to humankind, especially as our world population is expanding to over 6 billion people (38). As researchers focus more attention on the molecular mechanisms of plant nutritional physiology and biochemistry, as well as the variation that currently exists in our germplasm reserves, we in the plant science community will be in position to contribute significantly to the improvement of our plant-based food supply. However, decisions will have to be made regarding which nutrients to target and which crops to modify, such that the greatest nutritional impact is achieved. Because these decisions will require an understanding of human physiology and food chemistry, strong interdisciplinary collaborations will be needed among plant scientists, human nutritionists, and food scientists.

Improvement strategies can and should be developed now for the established, essential nutrients, as long as attention is paid to the upper safe limit of intake for each nutrient (see Table 1). However, regarding many of the nonessential phytochemicals with putative health benefits, more information is needed on their bioavailability and dose dependency, and an identification
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of specific molecular compounds having health efficacy is required, before plant improvement strategies should be pursued (51). Plant scientists can assist human nutritionists in this arena by providing stable isotope-labeled plant material to determine the bioavailability and subsequent metabolism of phytochemicals from whole foods (50). Additionally, efforts to identify cultivars or mutants with varied phytochemical composition can provide unique materials to be used in clinical investigations, such that the health-promoting activity of a single compound, or a class of compounds, can be deciphered.

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