Improving iron, zinc and vitamin A nutrition through plant biotechnology

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Recent understanding of plant metabolism has made it possible to increase the iron, zinc and β-carotene (provitamin A) content in staple foods by both conventional plant breeding and genetic engineering. Improving the micronutrient composition of plant foods may become a sustainable strategy to combat deficiencies in human populations, replacing or complementing other strategies such as food fortification or nutrient supplementation.

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Abbreviations
GGPP geranylgeranyl diphosphate
RE retinol equivalent

Introduction

In developing countries, problems of politics, distribution and poverty create food shortages and undernutrition. Many populations survive largely on plant-based diets with little or no meat or dairy products. Monotonous consumption of cereal staples, roots or pulses, in the absence of animal tissue, can lead to deficiencies of essential vitamins and minerals. Despite improvements over the past 50 years, over 2 billion people, one-third of the world's population, suffer from vitamin A, Zn and/or Fe deficiencies [1]. The global population, which reached 6 billion in late 1999, is estimated to climb to 8.3 billion in 2020. Most of this increase will occur in the cities of the developing world [2•] where the prevalence of these micronutrient deficiencies, and their associated morbidity and mortality, is highest.

During the 20th century, conventional breeding produced vigorous new plant varieties and hybrids that substantially increased yields and harvest stability, and improved nutrition. But, ever-increasing populations and changing demographics in the developing countries means the struggle for food security is far from over. To meet the macronutrient and micronutrient needs of over 8 billion people by the end of the coming quarter century, it is likely that both conventional crop technology and biotechnology will be needed.

Most of the research on plant breeding over the past few decades has concentrated on increasing resistance to environmental stresses, pests and pathogens [3]. Genetic engineering has even been used to improve the sensory appeal of agricultural products, such as tomatoes [4•]. However, the recent application of plant biotechnology to improve the nutritional content of staple food crops has perhaps the greatest potential to benefit global health [5,6,7••,8••]. Because poverty limits food access for much of the developing world's population, it is important that affordable staple foods be as nutritious as possible.

It may be possible to engineer synthetic pathways for many of the vitamins into plants, once the pathways are known and the corresponding genes have been cloned [9]. For example, *Arabidopsis* seed, like most oilseed crops, contains a high proportion of γ-tocopherol, which has only 10% of the vitamin E activity of α-tocopherol. Expression of the synthetic enzyme γ-tocopoherol methyltransferase in *Arabidopsis* seed resulted in the conversion of the large pool of γ-tocopherol to α-tocopherol with a corresponding 10-fold increase in vitamin E activity [10]. Improving the mineral content of plants, however, presents a different set of challenges. Unlike vitamins, which are synthesized by the plants themselves, plants must take up and store essential minerals from the soil. In this review, we focus on recent work with vitamin A and the minerals Fe and Zn.

Vitamin A

Half of the world's population eat rice (*Oryza sativa*) daily and depend on it as their staple food. Rice, however, is a poor source of many essential micronutrients and vitamins. In Southeast Asia, 70% of preschool children suffer from vitamin A deficiency [11]. Improved vitamin A nutrition could prevent 1 to 2 million deaths each year among children aged 1–4 years [11]. In tropical areas, rice is typically milled to remove the oil-rich aleurone layer to reduce rancidity during storage. The remaining edible portion, the endosperm, like the bran, lacks provitamin A (the plant carotenoids that are vitamin A precursors). Recently, Ye *et al.* [7••] have used genetic engineering techniques to produce rice grains containing β-carotene, the major precursor of vitamin A.

Immature rice endosperm can synthesize the intermediate compound geranylgeranyl diphosphate (GGPP). GGPP can be used to produce phytoene, an uncolored carotene, by expressing the enzyme phytoene synthase [12]. The synthesis of β-carotene from phytoene requires complementation with three additional plant enzymes: phytoene desaturase and β-carotene desaturase, each catalysing the introduction of two double bonds, and lycopene β-cyclase, encoded by the *lcy* gene. Ye *et al.* [7••] inserted the β-carotene biosynthetic pathway into rice endosperm via

Agrobacterium-mediated introduction of the genes for these enzymes; the genes for phytoene synthase, phytoene desaturase and lycopene β-cyclase were obtained from daffodil and the gene for carotene desaturase was of bacterial origin.

The simultaneous introduction of these genes was a major technical advance. Most traits engineered to date have only required the introduction of a single gene into the plant. Moreover, Ye *et al.* [7••] successfully introduced two of the genes (encoding phytoene synthase and phytoene desaturase) on a single construct that did not have a selectable marker. They did this by simultaneously introducing a second construct, which carried another of the genes of interest (lycopene β-cyclase) as well as a selectable antibiotic resistance gene. This approach might enable the investigators to segregate the antibiotic resistance gene away from the phytoene synthase and phytoene desaturase genes, avoiding a major concern of opponents to genetically modified crops [13].

The β-carotene was expressed in the grain at 1.6–2.0 μg/g fresh weight. Assuming that 6 μg β-carotene is converted to 1 μg retinol equivalents (REs) [14], 1.2 kg of 'golden' rice per day would be necessary to provide all the recommended vitamin A intake of a 4–8 year old child (400 μg RE). Therefore, even a relatively high rice intake of 200 g/day would provide only 70 μg RE or some 20% of the recommended vitamin A intake for this age group. The key question, however, is the bioavailability to man of the newly introduced β-carotene. Although the conversion factor to vitamin A for synthetic β-carotene and β-carotene in fruits is approximately 6:1, the conversion factor in vegetables, such as spinach, may be as low as 24:1 owing to poor release of the carotenoid during digestion [15]. The β-carotene level of this first transformation appears relatively low, when true-breeding lines are available it will be possible to more accurately determine levels of each type of carotenoid.

Whether the production of carotenoids in rice endosperm will cause adverse metabolic effects in field testing is unknown. Shunting the precursor GGPP to β-carotene production might decrease levels of other compounds whose synthesis is dependent on GGPP. For example, tomatoes engineered to produce more phytoene exhibit signs of dwarfism, owing to a 30-fold reduction in gibberellic acid, a plant hormone that shares the precursor GGPP with phytoene [13]. However, tomato plants express phytoene synthase in all tissues, whereas the rice of Ye *et al.* expresses it only in the endosperm, because the inserted genes are placed under the control of endosperm-specific promoters. This may reduce the potential for metabolic disturbance throughout the plant. Other recent examples of plant engineering of the terpenoid pathway to increase carotenoids in plant foodstuffs include manipulation of the later steps of the plastid terpenoid pathway to produce transgenic canola (*Brassica napus*) seed [16] and tomato (*Lycopersicon esculentum*) [17••]. For all of these foods, it remains to be demonstrated whether the provitamin A will be bioavailable in human studies.

Iron and zinc

Fe and Zn deficiencies are widespread in developing countries, where it is estimated that 40–45% of school-age children are anemic; approximately 50% of this anemia results from Fe deficiency [18]. Fe and Zn deficiencies weaken immune function and may impair growth and development. A major etiologic factor is the low bioavailability of Fe and Zn from diets based on staple cereals and legumes that are high in phytic acid, an absorption inhibitor [19]. There are several potential approaches to increasing the bioavailability of Fe and Zn in plant crops, including conventional breeding and genetic engineering [6].

Plant-breeding strategies

For staple crops, thousands of different genotypes of rice, wheat, maize, beans and cassava exist, and genotype influences the nutrient content of the plant and seeds [20]. In a recent screening of germ plasm, a high variation in the Fe and Zn content was reported in the edible parts of staple foods (wheat, bean, cassava, maize, rice and yam) between cultivars of a given species. For example, the Fe content of wheat cultivars varied from 25–56 mg/kg and the Zn content ranged from 25–64 mg/kg [6]. Moreover, the amounts of Fe and Zn in different rice and bean seeds appear to be highly correlated with each other [21,22]. However, the speciation and bioavailability of the minerals is not known. Research groups are searching for the genes determining high Fe and Zn levels, and several candidate genes have been identified in wheat on chromosomes 6A and 6B [23].

King *et al.* [24•] recently compared Fe and Zn bioavailability in humans for two varieties of the common red bean: an Fe- and Zn-rich genotype and a lower density genotype. The bioavailability of Fe and Zn from both varieties was low, about 1.5% and 13%, respectively. The 40% increase in Fe content in the high-density beans did not improve the amount of Fe absorbed. The 75% increase in Zn content was associated with a 40% increase in total absorbed Zn. The high phytate:mineral ratios of both genotypes probably lowered their mineral bioavailability. Reducing the phytate content of nutrient-dense plants might be necessary in order for these varieties to have a positive impact on human nutrition.

Using genetic screening, Raboy [25••] has identified several genotypes of maize, barley and rice that are low phytic acid (*lpa*) mutants. In these mutants, although the total phosphorus content of the seeds is unchanged, the phytic acid phosphorus is reduced by 55 to 66%. One of these maize mutants, *lpa1-1*, was used to develop inbred lines of isogenic hybrids. Compared with normal hybrids, few differences in agronomic characteristics were found, except for a 6% yield loss. Growth studies in chickens have found increased weight gain in chickens fed with a low phytic acid corn mutant versus normal hybrids [25••]. The

impact of low phytic acid maize consumption on Fe and Zn bioavailability has been tested in humans. Fe bioavailability was 49% greater from tortillas made with low phytic acid maize compared with wild-type maize (Fe absorption 8.2% versus 5.5%) [26]. Other studies, however, have shown that the phytic acid content of soy and wheat flour must be reduced by ≥90% to achieve a meaningful twofold increase in Fe absorption [27]. Adams *et al.* [28••] measured Zn bioavailability from low phytic acid maize versus wild-type maize. Mean Zn absorption from polenta prepared with the low phytic acid maize was 78% greater than for wild-type maize. These studies demonstrate that substitution of a low phytate variety may improve the bioavailability of these two trace minerals, but the impact on Fe nutrition in particular is uncertain. Combining cross-breeding techniques for developing low phytic acid plants with selection of highly nutrient-dense seeds could be an effective method for improving mineral nutrition.

Genetic engineering strategies

There are several genetic engineering approaches that could be used to increase the Fe content and bioavailability in plant foods. The Fe content could be increased by increasing soil Fe uptake or Fe storage within the edible plant [29]. Increasing the levels of siderophores, chelating agents, reducing agents, enzymes, and transporter proteins in roots could increase Fe uptake. Samuelsen *et al.* [30] recently reported on a transgenic tobacco plant expressing a yeast ferric reductase gene, which increased leaf Fe by 50%. Similarly, increasing the concentration of the storage proteins phytoferritin and metallothionein could increase the content of Fe and Zn, respectively. Goto *et al.* [31] reported a twofold to threefold increase in Fe in transgenic rice expressing the soybean ferritin gene. It may also be possible to introduce heat- and acid-resistant phytases into cereal grains. These phytases could withstand cooking and degrade phytic acid in the gut during digestion, thereby increasing absorption of Fe and Zn [32]. Finally, another option would be to introduce Fe absorption enhancers, such as ascorbic acid, cysteine-containing peptides or hemoglobin, into the edible plant tissues [33]. However, the Fe level of hemoglobin (0.3%) is probably too low to be a meaningful option, and the biosynthetic pathway for ascorbic acid in plants remains to be elucidated [34].

Although increasing the ferritin content of plants is likely to be the most realistic approach, ferritin Fe from animal sources is only 5–40% as well absorbed as ferrous sulfate [35], and absorption of plant ferritin has not been tested. Decreasing phytate in seeds is limited by the need for phytate during germination. Also, widespread introduction of phytases could increase Fe absorption in population groups, such as adult men and postmenopausal women, that already have ample body stores of Fe. This may be undesirable in that excess body Fe stores have been linked to several chronic diseases, including cardiovascular disease and cancer [36].

Lucca *et al.* [8••] reported on a combination of three genetic engineering approaches for improving the bioavailability and level of Fe in rice grains. Firstly, a thermostable phytase from *Aspergillus fumigatus*, active over the pH range found in the gastrointestinal tract, was introduced using an Agrobacterium-mediated transformation. The phytase had been developed to withstand food processing [37]. In addition, a ferritin gene from *Phaseolus vulgaris* was introduced. Finally, as cysteine peptides are considered to be enhancers of Fe absorption, the endogenous cysteine-rich metallothionein-like protein in rice was overexpressed. All three genes were expressed in the endosperm. The phytase activity increased sevenfold to a concentration similar to that of rye, which is the highest of the cereal grains. Phytic acid in uncooked rice was completely degraded during a simulated intestinal digestion, however, the phytase was not as thermostable after expression in the rice endosperm and was destroyed when the rice was cooked. The Fe content, however, was increased from 10 to 22 μg/g and the cysteine residue content increased from 45 to 320 mg/g protein [8••]. The influence of these changes on Fe bioavailability remains to be investigated.

Conclusions

Before modified crops can be incorporated into agricultural systems to improve nutrition, several important background questions need to answered. Is there a demonstrated and clear need? Is the food to be altered important for the population and will it be delivered reliably and continually? Will it be acceptable? What are the biological barriers to genetic improvement and are there other more cost-effective approaches available? Considering these issues before beginning an engineering or breeding program is critical to a program's potential for success [38]. After the modified crop is field-tested, its nutritional efficacy needs to be carefully determined using *in vitro* and *in vivo* tests before it is added to the food supply of a target population. After introduction, the nutrition and health impact, as well as the environmental impact, needs to be monitored. These implementation steps require a coordinated effort of policy-makers, plant scientists, nutritionists and economists.

Significant progress has been made since the mid-1980s in the molecular understanding of many nutritionally related plant pathways and in the use of cloned genes to engineer plant metabolism. A gap remains, however, between the ability to clone and manipulate individual genes, and the understanding of how they integrate into and impact on plant metabolism [39]. Preliminary studies indicate that Fe, Zn and β-carotene levels can be increased in staple foods by plant breeding and/or genetic engineering. The challenge is now to show that they can be increased to nutritionally useful levels and to demonstrate that the additional micronutrient is bioavailable. This has not yet been evaluated. Despite this, these approaches show great promise as sustainable ways to combat micronutrient deficiencies.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Food and Agriculture Organization of the United Nations (FAO): *Preventing Micronutrient Malnutrition. A Guide to Food-Based Approaches.* Washington DC: International Life Science Institute; 1997:105.

2. • Miflin B: Crop biotechnology. Where now? *Plant Physiol* 2000, 123:17-28.
A comprehensive update and reflections on the future of plant biotechnology.

3. Borlaug NE: Ending world hunger. The promise of biotechnology and the threat of antiscience zealotry. *Plant Physiol* 2000, 124:487-490.

4. • Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor Y, Nam K, Amar O, Lastochkin E, Larkov O, Ravid U *et al.*: Enhanced levels of the aroma and flavor compound ***S***-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. *Plant Physiol* 2001, 127:1256-1265.
A new genetic engineering approach to improve the sensory appeal of tomatoes by increasing *S*-linalool content.

5. Graham RD, Senadhira D, Beebe S, Iglesias C, Montasterio I: Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. *Field Crops Res* 1999, 60:57-80.

6. Frossard E, Bucher M, Mächler F, Mozafar A, Hurrell R: Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J Sci Food Agric* 2000, 80:861-879.

7. •• Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, Potroykus I: Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* 2000, 287:303-305.
These researchers inserted the β-carotene biosynthetic pathway into rice via Agrobacterium-mediated introduction of the genes for three enzymes. Rice grains containing increased levels of β-carotene were produced.

8. •• Lucca P, Hurrell R, Potrykus I: Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *Theor Appl Genet* 2001, 102:392-397.
Three genetic engineering approaches were combined to improve the bioavailability and level of Fe in rice grains.

9. DellaPenna D: Nutritional genomics: manipulating plant micronutrients to improve human health. *Science* 1999, 285:375-379.

10. Shintani D, DellaPenna D: Elevating the vitamin E content of plants through metabolic engineering. *Science* 1998, 282:2098-2100.

11. Unicef: Vitamin A global initiative. URL: www: unicef.org/vitamina

12. Cunningham FX, Gantt G: Genes and enzymes of carotenoid biosynthesis in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1998, 49:557-583.

13. Guerinot ML: Perspectives: plant biology. The green revolution strikes gold. *Science* 2000, 287:241-243.

14. National Research Council: *Recommended Dietary Allowances.* 10th edition, Washington DC: National Academy Press; 1989: 78-91.

15. Institute of Medicine: β-Carotene and other carotenoids. In *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids.* Washington, DC: National Academy Press; 2000:325-382.

16. Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY: Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. *Plant J* 1999, 20:401-412.

17. •• Römer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM: Elevation of the provitamin A content of transgenic tomato plants. *Nat Biotechnol* 2000, 18:666-669.
Engineering of the late terpenoid pathway to increase carotenoid content in tomato.

18. World Health Organization/United Nations Children's Fund/United Nations University: IDA: Prevention, assessment and control. In *Report of a joint WHO/UNICEF/UNU consultation*. Geneva: WHO; 1998:1-9.

19. Hurrell RF: Fortification: overcoming technical and practical barriers. *J Nutr* 2002, in press.

20. Graham RD, Welch RM: *Breeding for Staple Food Crops with High Micronutrient Density.* Washington, DC: International Food Policy Research Institute; 1996:1-79.

21. Beebe S, Gonzalez AV, Rengifo J: Research on trace minerals in the common bean. *Food Nutr Bull* 2000, 21:387-391.

22. Gregorio GB, Senadhira D, Htut H, Graham RD: Breeding for trace mineral density in rice. *Food Nutr Bull* 2000, 21:382-386.

23. Cakmak I, Ozakan H, Braun JJ, Welch RM, Römheld V: Zinc and iron concentrations in seeds of wild, primitive and modern wheats. In *Improving Human Nutrition through Agriculture: the Role of International Agriculture Research.* Workshop hosted by the International Rice Research Institute, Los Banos, Philippines and organized by the International Food Policy Research Institute, 5–7 October, 1999.

24. • King JC, Donangelo CM, Woodhouse LR, Mertz SD, Shames DM, Viteri FE, Cheng Z, Welch RM: Measuring iron and zinc bioavailability in humans. *Food Nutr Bull* 2000, 21:434-439.
Fe and Zn bioavailability in humans were compared for two varieties of the common red bean: an Fe- and Zn-rich genotype and a lower density genotype.

25. •• Raboy V: Low-phytic-acid grains. *Food Nutr Bull* 2000, 21:423-427.
This work describes the identification of low phytic acid (*lpa*) maize mutants and the development of inbred lines of isogenic hybrids.

26. Mendoza C, Viteri FE, Lönnerdal B, Young KA, Raboy V, Brown KH: Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *Am J Clin Nutr* 1998, 68:1123-1127.

27. Hurrell RF: Influence of vegetable protein sources on trace element and mineral bioavailability. *J Nutr* 2002, in press.

28. •• Adams C, Raboy V, Krebs N, Westcott J, Lei S, Hambidge KM: The effect of low-phytic acid corn mutants on zinc absorption. *FASEB J* 2000, 14:A510.
This study measured Zn bioavailability from low phytic acid maize; Zn absorption with the low phytic acid maize was 78% greater.

29. Grusak MA, DellaPenna D: Improving the nutrient composition of plants to enhance human nutrition and health. *Annu Rev Plant Physiol Plant Mol Biol* 1999, 50:133-161.

30. Samuelsen AI, Martin RC, Mok DW, Mok MC: Expression of the yeast FRE genes in transgenic tobacco. *J Plant Physiol* 1998, 118:51-58.

31. Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F: Iron fortification of rice seeds by the soybean ferritin gene. *Nat Biotechnol* 1999, 17:282-286.

32. Hurrell RF: Modifying the composition of plant foods for better human health. In *Crop Science: Progress and Prospects*. Edited by Nösberger J, Geiger HH, Struik PC. New York: CABI Publishing; 2001:53-64.

33. Fairweather-Tait SJ, Hurrell RF: Bioavailability of minerals and trace elements. *Nutr Res Rev* 1996, 9:295-324.

34. Davey MW, Montagu MV, Inze D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJ, Strain JJ, Favell D, Fletcher J: Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J Sci Food Agric* 2000, 80:825-860.

35. Skikne B, Fonzo D, Lynch SR, Cook JD: Bovine ferritin iron bioavailability in man. *Eur J Clin Invest* 1997, 27:228-233.

36. Kang JO: Chronic iron overload and toxicity: clinical chemistry perspective. *Clin Lab Sci* 2001, 14:209-219.

37. Pasamontes L, Haiker M, Wyss M, Tessier M, van Loon AP: Gene cloning, purification, and characterization of a heat-stable phytase from the fungus ***Aspergillus fumigatus***. *Appl Environ Microbiol* 1997, 63:1696-1700.

38. Bliss FA: Nutritional improvement of horticultural crops through plant breeding. *Hortic Sci* 1999, 34:1163-1167.

39. DellaPenna D: Plant metabolic engineering. *Plant Physiol* 2001, 125:160-163.