ISB NEWS REPORT
AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY

JANUARY 2010

SPECIAL ISSUE:
BIOFORTIFICATION OF PLANTS THROUGH GENETIC ENGINEERING

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THE ACCUMULATION OF NOVEL OMEGA-3 FATTY ACIDS IN TRANSGENIC PLANTS

Johnathan Napier, Noemi Ruiz-Lopez, Tianbi Li, Richard Haslam, Olga Sayanova

The advent of plant genetic engineering over two decades ago hastened new thinking on breeding crops with novel traits. In particular, the concept of modifying seed oil composition to alter and improve its enduse was exemplified by pioneering studies by researchers at Calgene Inc. (Davis, CA), who modified plant oils with "gain-of-function" transgenes.[1] Through those and similar studies, the "designer oilseed" concept was born—the ability to manipulate and modify, in a predictable manner, the composition of a plant oil.[2]

Fatty acids found in the plant kingdom collectively display considerable chemical diversity, in excess of 400 different fatty acids, with most of the so-called unusual fatty acids compartmentalized in seed storage oils.[2,3] And although many unusual fatty acids in plants have potentially useful applications, ranging from industrial biolubricants and petrochemical replacements to human nutrition, most wild plant species are unsuitable for modern agricultural practices. Use of genetic engineering to transfer the "trait" for unusual fatty acids between species is limited by the identification of the gene(s) underlying the unusual fatty acid trait.[1-3]

Enormous progress has been made in understanding genetic components required for the biosynthesis and modification of fatty acids in plants, thus facilitating the identification of sequences responsible for unusual fatty acid accumulation. Perhaps surprisingly, many early attempts to engineer the fatty acid composition of transgenic plants produced much lower levels of target lipids than found in native species.[2] Such observations elucidate the complexity of plant lipid metabolism. Consequently, employing iterative "learning–by-doing" approaches to engineering novel oil traits is advancing real progress in this area.[4]

Omega-3 Fatty Acids and Human Health

One promising area of research pertains to the production of omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), the so called fish oils, in transgenic plants. There is compelling evidence for the health benefits of a diet rich in fish oils, e.g., in providing for optimal development of the unborn child and protection against cardiovascular disease. However, animals have only a limited capacity to synthesize these fatty acids, so dietary intake is important.

Unfortunately, a number of factors limit our consumption of omega-3 polyunsaturated fatty acids.[3-5] First, the over-exploitation of wild fish stocks has reduced their sustainability, exacerbated by the increasing demands of aquaculture for fish oils and meal. Second, the environmental pollution of marine food webs has raised concerns over the ingestion of toxic substances such as heavy metals, PCBs, and dioxins. Finally, shifts in food production methods and changes to the historical patterns of food consumption have culminated in diets dominated by omega-6 fatty acids, as opposed to the (more health-protective) omega-3s.

To address these issues, we and others are focusing on producing omega-3 long-chain polyunsaturated fatty acids in transgenic plants to provide a safe and sustainable source of

these important oils.[3-5] Therefore, the goal is not the direct replication of the fatty acid profile found in marine microbes (the primary biosynthetic source of omega-3 LC-PUFA) or fish, but rather the nutritional enhancement of vegetable oils by the inclusion of specific marine fatty acids not normally synthesized by higher plants. In such a scenario, the dietary intake of these healthy fats would be achieved by consumption of omega-3 LC-PUFA-enhanced vegetable oils, without a need for increased consumption of fish or supplements.

Transgenic Synthesis of Omega-3s

Considerable effort has focused on the transgenic synthesis in plants of two particular omega-3 LC-PUFAs: eicosapentaenoic acid (EPA20:5$\Delta^{5,8,11,14,17}$) and docosahexaenoic acid (DHA; 22:6$\Delta^{4,7,10,13,16,19}$). EPA and DHA are the predominant fatty acids in fish oils, and there is strong evidence based on epidemiological, clinical, and genetic studies of their cardiovascular system-protective role. However, the conversion (through genetic engineering) of a plant C18 fatty acid such as α-linolenic acid (ALA; 18:3$\Delta^{9,12,15}$) to EPA requires three separate enzyme activities (two desaturations interspersed with a two-carbon chain elongation) and conversion of ALA to DHA requires five different enzyme activities (desaturation, elongation x2, desaturation).

Despite recent progress in achieving useful levels (10 – 25% of total fatty acids) of EPA in transgenic seed oils,[5,6] coordination of the expression and activity of multiple enzyme activities that act in a sequential manner is an on-going metabolic engineering challenge: this is even more true for DHA, where the levels achieved in transgenic plants are still relatively low (<5% of total fatty acids).[5,6] Consequently, attention has also been drawn to the C18 omega-3 fatty acid stearidonic acid (SDA; 18:4$\Delta^{6,9,12,15}$), which is synthesized by the a single (Δ6-)desaturation reaction of ALA[7]. SDA shares many of the health benefits of EPA, presumably because it is a biosynthetic intermediate of that fatty acid; thus, consumption of SDA boosts EPA levels by bypassing our limited endogenous ability to desaturate ALA.

SDA is an example of an unusual plant fatty acid found in only a few species, predominantly members of the Boraginaceae family. Only one species (*Echium* spp.) is grown commercially for SDA, though this may change given increasing interest in this fatty acid. It should also be noted that *Echium* oil not only contains ~14% SDA, but also has significant levels of the omega-6 C18 γ-linolenic acid (GLA; 18:3$\Delta^{6,9,12}$). GLA, like other omega-6 fatty acids, does not deliver the health benefits seen with SDA or other omega-3 PUFAs, such as EPA and DHA.

Production of Stearidonic Acid in Transgenic Plants

On paper, engineering plants to accumulate high levels (>10%) of total fatty acids should be straightforward through the seed-specific expression of a suitable Δ6-desaturase isolated from borage or similar species. However, enhanced expression of Δ6-desaturase also produces the co-synthesis of GLA, since most Δ6-desaturases have no substrate preference for omega-6 (LA) or omega-3 (ALA).

In earlier studies, we identified Δ6-desaturases from *Primula* spp. that showed strong substrate preference for ALA; thus, by using the *Primula* Δ6-desaturase, SDA can be generated without producing GLA. Previous attempts to generate transgenic plants high in SDA but low in GLA had some success, most notably by Eckert et al.,[8] who co-expressed borage Δ6-desaturase with a Δ15-desaturase (to convert GLA to SDA) in transgenic soybean. This resulted in an SDA concentration between 10% – 29%, but the soybeans still contained significant GLA (7.2% – 12.4%).

PUBLISHED BY

Information Systems for Biotechnology

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We sought to avoid the complications of using multiple enzyme activities by expressing the *Primula vialii* Δ6-desaturase in transgenic Arabidopsis and linseed, under control of a seed specific promoter.[7] Thus, the same construct was introduced by Agrobacterium-mediated transformation into either a model plant or an actual oilseed crop. This very straightforward experiment immediately provided some interesting results, most strikingly, the disparity in the achieved levels of SDA between the two plant species: whereas linseed accumulated ~12% SDA as part of total seed fatty acids, Arabidopsis seed oils only contained ~3% SDA.[7] Attempts to elevate Arabidopsis SDA, by increasing the amount of substrate ALA to equal that found in linseed, also failed to improve this low yield. Thus, it seems clear that even simple transgenic traits for modified plant lipid metabolism are context-dependent, meaning that the configuration of endogenous biochemical pathways for seed oil synthesis, modification, and accumulation significantly influences the efficacy of any transgene-derived heterologous activity.

"... the "cut and paste" method of using heterologous transgene-derived acyl-modifying activities needs to incorporate the additional qualities of the host ..."

The acyl composition of plant oils varies markedly between species, even though they share a common set of biosynthetic enzymes. Subtle differences in the level and temporal expression of enzyme activities during seed development, which varies from species to species,[3,4] largely determine the final composition of any given seed oil.[7] Therefore, the "cut and paste" method of using heterologous transgene-derived acyl-modifying activities needs to incorporate the additional qualities of the host, which requires a detailed biochemical characterization of the pathways of oil synthesis.

Linseed as Novel SDA-Enriched Omega-3 Crop

Though Arabidopsis proved a poor accumulator of SDA, linseed accumulates levels of SDA which are equivalent to the native *Echium* species.[7] Perhaps more importantly, the ALA-specific Δ6-desaturase derived from *Primula* synthesized SDA without any co-synthesis of omega-6 GLA. Thus, transgenic SDA-containing linseed differed from both *Echium* and transgenic soybean oil by being essentially devoid of GLA.[7,8] Detailed analysis of different lipid classes in developing and mature linseed lines confirmed the preferential accumulation of SDA in triacylglycerols (neutral storage lipids) as opposed to phospholipids (the actual site of synthesis of SDA).[7] Thus, linseed efficiently channeled SDA from phospholipids to triacylglycerols through an acyl-CoA-independent pathway. This result confirms our observations from earlier attempts to synthesize EPA in transgenic linseed in which C20 LC-PUFAs generation was blocked by the absence of substrates (such as SDA) in the acyl-CoA pool.[9]

Transgenic linseed engineered to accumulate SDA is a potentially valuable novel oilseed. The oil of this SDA-enriched linseed contains 60% omega-3 fatty acids, of which approximately one quarter is SDA (14%). In addition, not only are levels of the native omega-6 LA relatively low (12%), the oil contains only trace levels of omega-6 GLA (0.3%). The absence of GLA is important, as this Δ6-desaturated fatty acid can (in animals) be converted to the C20 omega-6 arachidonic acid, which in turn is a precursor for pro-inflammatory eicosanoids. Thus, for "heart-healthy" applications, transgene-derived SDA-enriched linseed oil may be superior for enhanced omega-3 nutrition.

In conclusion, these studies illustrate both the power of genetic engineering to generate novel oil traits and the need for a deeper understanding of plant lipid metabolism. It is hoped that the combination of these factors will, in the near future, deliver further beneficial oilseed crops carrying new transgene-derived traits, as well help to improve knowledge of the underlying biochemical processes.

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BIOFORTIFICATION OF VITAMIN B_6 IN SEEDS

Hao Chen, Liming Xiong

The Versatile Functions of Vitamin B_6

Water-soluble pyridoxine, pyridoxal, and pyridoxamine are collectively called vitamin B_6. These vitamers are remarkably versatile, providing essential functions in a large number of biological reactions. Approximately four percent of all classified enzymatic activities are pyridoxal 5'-phosphate-dependent\. Pyridoxal 5'-phosphate (PLP), the coenzyme form of vitamin B_6 that can be converted from pyridoxine, pyridoxal, and pyridoxamine, is required by numerous enzymatic reactions, including transaminations, aldol cleavages, beta- or gamma-eliminations or replacements, decarboxylations, and racemizations that are involved in the biosynthesis and/or catabolism of amino acids, structural compounds, neurotransmitters, histamine, hemoglobin, lipids, and carbohydrates.[1] Recently, vitamin B_6 has also been shown to function as a potent antioxidant, equivalent to vitamins C and E, and is particularly active in quenching singlet oxygen and the superoxide anion. Moreover, PLP is involved in enhancing or suppressing the expression of certain genes. PLP conjugates to a lysine residue in the nuclear repressor RIP140, and such conjugation is essential for RIP140's interaction with histone deacetylases, nuclear retention, and subsequent transcriptional repression.

Vitamin B_6 provides a great number of health benefits and is essential for all cellular organisms. It is particularly known in the medical field for its involvement in more bodily functions than any other single nutrient. Vitamin B_6 is required for the maintenance of mental as well as physical health[2] and is essential for normal brain development and function. Vitamin B_6 is especially important for maintaining healthy muscle cells, absorption of vitamin B_{12}, synthesis of vitamin B_3, and the production and proper function of red blood cells and cells of the immune system.[3] Moreover, vitamin B_6 can inhibit platelet aggregation, lower blood pressure, and protect against the development of diabetic neuropathy.

Consequently, vitamin B_6 deficiency will result in high levels of homocysteine, muscle weakness, skin inflammation, nervousness, irritability, fatigue and sleepiness, difficulty in concentration, and short-term memory loss. Deficiency of this vitamin has thus been associated with depression, epilepsy, impaired cognitive functions, Alzheimer's disease, cardiovascular diseases, and different types of cancer.[3] Although primary vitamin B_6 deficiency is rare in developed countries, it is estimated that 14% of people in America fall below the estimated average requirement[4] for vitamin B_6. In addition, certain populations such as infants and children, elderly people, and people who consume excessive amounts of alcohol or smoke cigarettes are at a higher risk of vitamin B_6 deficiency.

Seeds Are a Good Target for Elevating Vitamin B_6

The vitamin B_6 biosynthesis pathway, in which pyridoxine is synthesized from 1-deoxy-D-xylulose-5-phosphate and 4-phosphohydroxy-L-threonine through Pdx2A and Pdx2J, is well-characterized in eubacteria such as *E. coli*. Recently, another distinctive vitamin B_6 biosynthesis pathway was discovered in fungi, archaeabacteria, certain eubacteria, and plants. In these organisms, pyridoxal 5'-phosphate is directly synthesized from glutamine and ribose 5-phosphate or ribulose 5-phosphate through a highly conserved bi-enzyme complex consisting of PDX1, the acceptor/synthase, and PDX2, the glutaminase domain. In contrast to bacteria, fungi, and plants, which have the ability to synthesize their own supply of vitamin B_6 *de novo*, animals cannot synthesize the vitamin and need to obtain it from their diet. Unlike fat-soluble vitamins, which can be stored in the liver, water-soluble vitamin B_6 is not or barely stored in the body, and the excess is excreted through the urine. Therefore, animals need a continuous supply of the vitamin included in their daily diet. Since plants are the major source of vitamin B_6 for animals either directly or indirectly, it is of great interest to increase vitamin B_6 levels in plants for improved nutrition value.

Thus far, there is only one recent report of research to engineer vitamin B_6 content in plants. Herrero and Daub overexpressed *PDX1* and *PDX2* genes from the fungi *Cercospora nicotianae* in tobacco. However, only one of all transgenic plants tested had an ~21% increase in vitamin content. One problem with this heterologous expression system is that it suppressed endogenous *PDX* genes.[5]

In our study, we initially generated transgenic Arabidopsis using endogenous *PDX* genes under control of the CaMV 35S promoter. Although *PDX1* or *PDX2* transcript levels are greatly increased in these 35S promoter-driven overexpression lines, no dramatic increase in vitamin B_6 content was detected in either shoots or roots. It seems that vitamin B_6 homeostasis is more tightly regulated in these vegetative parts. In contrast to shoots and roots, a significantly higher amount of pyridoxine accumulated in the seeds of all these transgenic plants. Since the CaMV 35S promoter has a low activity during early embryogenesis, and its activity greatly decreases during seed development, we decided to over-express the *PDX* genes specifically in seeds using a seed-specific promoter. We made constructs consisting of the *PDX1* or *PDX2* cDNA under control of the Arabidopsis 12S seed storage protein gene promoter individually or in tandem and transferred them separately into wild-type Arabidopsis plants. We found that all lines have significantly increased pyridoxine and pyridoxamine contents in dry seeds. The total vitamin B_6 level in some transgenic lines is three times that of wild type.[6] Our results indicate that the seed is a suitable target organ for further engineering high levels of bio-available vitamin B_6.

Recently, a concern has been raised about the decline in nutrient quality of crops with increased yield, and their subsequent effect on nutrient quality of meat and dairy products when they are used as animal feed. Because seeds are relatively rich in vitamin B_6, the two-fold increase of the vitamin in engineered seeds demonstrated in our work is particularly valuable for the crop plants whose seeds are a major source of food and feed. In addition to the nutritional advantage, elevated vitamin B_6 in seeds may help seeds in combating the pathogenic fungus *Cercospora nicotianae*. This pathogen produces a strong singlet oxygen-generating toxin called cercosporin, and vitamin B_6 is indispensible for quenching the radicals from this toxin. In addition, enhanced vitamin B_6 levels in seeds may also inhibit lipid peroxidation induced by reactive oxygen species (ROS) and preserve the seed quality during the long-term storage.

It is expected that in the near future vitamin B_6 levels in seeds can be further increased by several other means once the biosynthesis and catabolism pathways as well as their regulation are better understood. For example, one may combine the use of mutant lines that contain elevated glutamine or other precursors of vitamin B_6 biosynthesis with PDX transgenic lines, employ other seed-specific promoters that are active during earlier stages of embryo and endosperm development, reduce the catabolism of the vitamers, and relieve possible end-production inhibition on the biosynthetic pathway.

"Our results indicate that the seed is a suitable target organ for further engineering high levels of bio-available vitamin B_6."

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IRON BIOFORTIFICATION OF RICE BY TARGETED GENETIC ENGINEERING

Christof Sautter and Wilhelm Gruissem

Rice plants have been developed that contain six times more iron in polished rice kernels. To accomplish this, two plant genes were transferred into an existing rice variety. In the future, high-iron rice could help to reduce iron deficiency in human nutrition, especially in developing countries in Africa and Asia. Moreover, engineered plants will be useful to study the regulation of iron homeostasis *in planta*.

Iron Malnutrition Is a Serious Problem

Iron deficiency is the most common and widespread nutritional disorder in the world. According to the World Health Organization, approximately two billion people suffer from iron deficiency—they tire easily, experience problems metabolizing harmful substances, and eventually suffer from anemia. Based on the recorded incidence of anemia, most preschool children and pregnant women in developing countries and at least 30 – 40% in industrialized countries are iron deficient.[1,2] In developing countries where rice is the major staple food (**Fig. 1**), children are particularly affected, as well as women during their fertile life period. Peeled rice, also called polished rice, does not have enough iron to satisfy the daily requirement, even if consumed in large quantities. Rice actually has a lot of iron, but only in the seed coat. Because unpeeled rice quickly becomes rancid in tropical and subtropical climates, the seed coat—along with precious iron—must be removed for storage. For many people, a balanced diet or iron supplements are often unaffordable. Moreover, iron is the most difficult mineral for food fortification, since the most soluble and absorbable iron compounds (e.g., $FeSO_4$) are unpalatable, and less soluble iron compounds are poorly absorbed.[3]

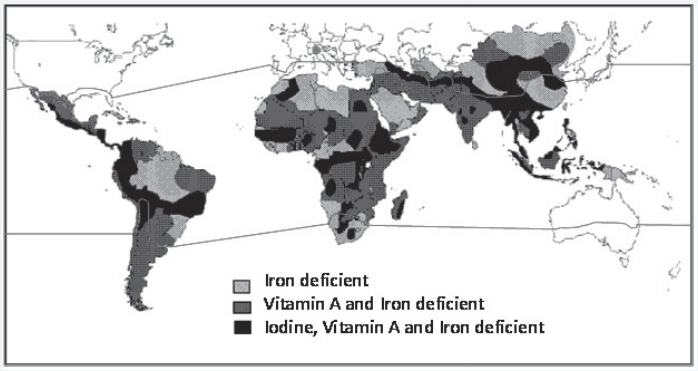

Figure 1. Correlation of risk distribution for different micronutrient malnutritions and rice growing area Iron malnutrition and rice area are coincident in large parts of the world. *[Data about risk of mirconutrient malnutrition is from Cornell University (www.css.cornell.edu/foodsystems). Data about area where rice grows – between the red lines – is from IRRI (www.irri.org/gis/ricedist/maps).]*

Conventional breeding and genetic engineering are promising alternative approaches for increasing the iron content of food crops. Until now, however, conventional breeding has only produced a very limited increase of iron in rice endosperm, most likely because seed iron content is a complex trait. Similarly, genetic engineering using single transgenes for iron transport or accumulation has not produced the expected increase, and whether gene transfer of transcription factors leads to the desired higher iron content without detrimental side effects is an open question.[4]

Two Plant Genes Help to Mobilize and Store Iron

We have recently succeeded in increasing the iron content in polished rice by transferring two plant genes into an existing japonica rice variety.[5] One gene encodes nicotianamine synthase, the enzyme that produces nicotianamine. Nicotianamine chelates iron temporarily and facilitates its transport in the plant. Nicotianamine synthase is expressed under a constitutive promoter. The second gene encodes the protein ferritin, which functions as a storage depot for up to four thousand iron atoms per protein molecule in both plants and humans. Since the ferritin gene is under the control of an endosperm-specific promoter, ferritin comprises a sink for iron in the center of the endosperm. The synergistic action of these two genes allows the rice plant to absorb more iron from the soil, transport it in the plant, and store it in the rice kernel. A third gene encoding phytase was also engineered into this rice line. Phytase degrades phytate, a compound that stores phosphate and binds divalent cations like iron and thus inhibits their absorption in the intestine.

Iron Content of Biofortified Line is Relevant

The genetically engineered lines expressing nicotianamine synthase, ferritin, and phytase (NFP-line) contain up to a 6.3-fold increase of iron in the endosperm of polished kernels as compared to wild type, and significantly more than the lines that contain only single genes, i.e., nicotianamine synthase (NAS) or ferritin (FER) (**Fig. 2**). This increase is independent of the iron concentration in the medium.[5] In contrast, iron content in leaves does not differ significantly, but increases concomitantly with iron content in the medium (**Fig. 3**). Laser ablation inductive coupled plasma mass spectroscopy (LA-ICP-MS)[6] confirmed the accumulation of additional iron in the endosperm of NFP lines.[5] **Figure 4** shows iron distribution by imaging micro-X-ray fluorescence spectroscopy.[5]

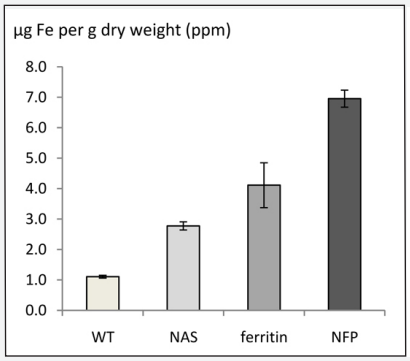

Figure 2. Iron concentration in polished seeds Concentration of iron in polished grains of wild-type (WT), NAS, ferritin, and NFP plants on 200µM iron supplied in the medium. Values are the means of four individual plants (± standard deviation). All values are significantly different ($P < 0.001$). Iron content is given as µg Fe per g dry weight (ppm).

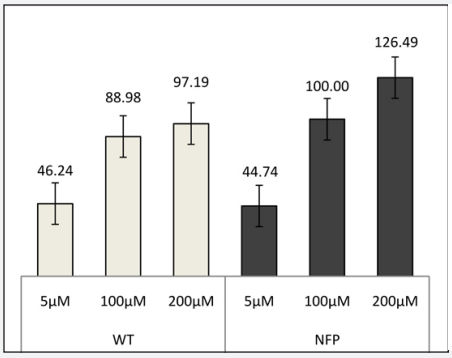

Figure 3. Iron concentration in leaves Concentration of iron in leaves of wild type (WT) and NFP plants as a function of external iron concentration in the medium, which was 5, 100 and 200µM. Values are the means of four individual plants (± standard deviation). Numbers above the bars indicate metal content in % relative to NFP 100 µM Fe.

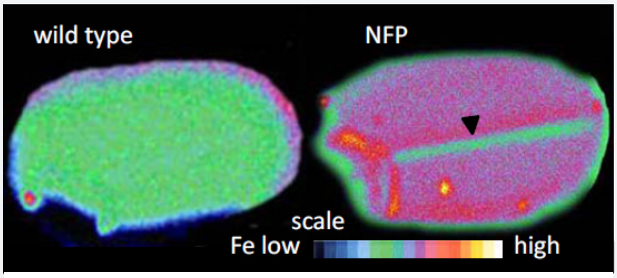

Figure 4. Localization of iron in grains Element-sensitive mapping of iron concentration in a representative WT and NFP grain, acquired using micro-X-ray fluorescence spectrometry. The color indicates iron concentration. The low iron line in NFP represents the laser ablation track, which is out of focus and therefore iron concentration appears to be reduced along this line (arrowhead).

There are no reports of bred or engineered rice varieties with a comparably high iron increase in endosperm. Field experiments and feeding studies are planned to test the agronomic performance and efficacy of high-iron rice lines. Previous nutritional studies, using a conventionally-bred rice variety with less than half the iron content of our NFP lines, demonstrate that the iron status of women can be improved by rice varieties containing about 3 µg Fe/g dry-weight (DW).[7] Our engineered rice line contains up to 7 µg Fe/g DW. Further increasing the iron content of our NFP rice lines from 7 µg to about 15-18 µg Fe/g DW would satisfy the iron requirement with one meal of rice a day. In order to achieve this, it might be sufficient to use high iron genotypes for genetic engineering, containing up to 4 µg Fe/g DW already in the starting material.[8]

Maintenance of Iron Homeostasis

One obstacle to iron biofortification of plants is the toxicity of iron when it accumulates to higher concentrations in cytoplasm. Plants therefore regulate the uptake and concentration of iron in their cells by altering nicotianamine concentration through expression or activity of nicotianamine synthase or a degrading enzyme, nicotianamine amino transferase (NAAT), in response to an iron-dependant signal. Constitutive expression of nicotianamine synthase in combination with ferritin in the endosperm increases iron in sink tissue, but does not change iron homeostasis in leaves, despite higher levels of nicotianamine.[5] Expression of the gene for nicotianamine-degrading NAAT is stimulated,[9] probably by higher levels of nicotianamine in leaves of NFP-plants (**Fig. 5**).

Studies are underway to monitor the expression of other endogenous rice genes involved in iron homeostasis. Understanding the regulation of these genes in our NFP lines will allow us to make further targeted improvements in iron content, e.g., by uncoupling regulated genes from product feedback, using appropriate promoters.

Distribution of High Iron Rice to Farmers Still Many Years Away

The NFP high-iron rice lines were phenotypically and physiologically normal under greenhouse conditions.[5] It is unlikely that the high-iron rice will negatively affect soil, because iron is the most prevalent metallic element on earth and very abundant in soil. Nevertheless, before these improved rice lines can be released, they must be extensively tested in the field for their agronomic performance, trait stability, and biosafety. The genes also need to be transferred to other widely-used japonica and indica varieties before they can be made available to farmers. The experience with high-vitamin A "Golden Rice," which was also developed at ETH Zurich in collaboration with researchers at the University of Freiburg (Germany), has shown that it takes many years before genetically engineered rice can actually be planted by farmers.[10] The regulatory hurdles and costs involved in making genetically modified plants available to agriculture and consumers are very high. In addition, appropriate seed distribution channels and agricultural production systems must be established before high-iron rice plants can be available to small-scale and self-sufficient farmers free of charge.

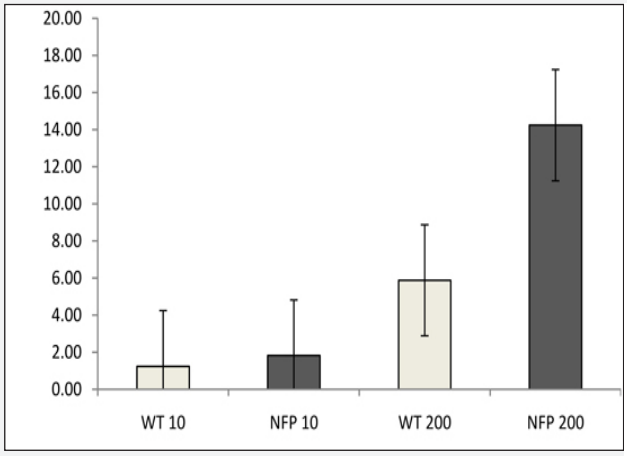

Figure 5. Relative mRNA abundance of NAAT in rice leaves
Relative mRNA abundance of nicotianamine amino transferase (NAAT) in leaves of wild-type (WT) and NFP plants at 10 µM Fe (10) and 200 µM external iron concentration in the medium.[8] Values are the means of four individual plants (± standard deviation). NFP 200 is significantly different (t-test) from all other values by an error probability lower than 0.01.

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FEEDING FUTURE POPULATIONS WITH NUTRITIONALLY COMPLETE CROPS

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Imagine a world in which no one goes hungry and no one is malnourished. It sounds too much to hope for when we look around the real world and see that up to half the population lacks access to a balanced diet.[1,2] The best way to provide adequate supplies of essential vitamins and minerals is to eat a varied diet, including fresh fruit and vegetables, fish, and dairy products. In the West, an adequate diet can be achieved by visiting the supermarket or grocery store, but in developing countries many people subsist on monotonous diets of staple cereals, such as rice, corn, and wheat. Although they provide calories, cereal grains are poor sources of most vitamins and minerals; a diet comprised mostly from cereals will address hunger, but not malnutrition. Micronutrient deficiency diseases are therefore rife in the developing world, causing millions of needless deaths and adding to miserable socio-economic conditions.[3]

Many strategies have been proposed to address nutrient deficiencies, including supplement distribution, fortification programs, and attempts to make crops more inherently nutritious.[4] Unfortunately, such programs have had limited success: first, because they require significant funds and a good organizational infrastructure, both of which tend to be lacking in developing countries; and second, because they rely on compliance from farmers and consumers. Fortification programs have been successful in some cases, e.g., salt iodization, but these are rare exceptions and merely shift the problem onto the remaining nutrient deficiencies. Biofortification is the most ambitious approach, as it attempts to address the problem at the source. For example, the levels of several mineral nutrients in crops can be improved by including mineral salts in fertilizers. As above, however, there has only been limited success, and only when there is a good infrastructure and enough money to pay for fertilizers. This excludes a significant proportion of the most malnourished people in the world, who cannot afford the technical measures to improve the nutrient content of their own crops.

A relatively new approach is to create novel crop varieties that are more nutritious, thereby removing the onus of compliance from producers and consumers alike. There is significant genetic variation in the quantity of some nutrients, so breeding crops and selecting those with higher levels of vitamins and minerals seems like a logical approach.[1] Unfortunately, trying to enhance nutrient levels by conventional breeding is a very long-term venture, particularly when the aim is to transfer nutrient-rich traits into locally-adapted breeding varieties. Even if this could be achieved in a reasonable time scale, the complexity of breeding for several different nutrients at once would be insurmountable, and some nutrients are simply not present at high enough levels to make breeding a viable option. Conventional breeding is therefore a dead end when constructing a visionary strategy for generating nutritionally complete cereal crops.

What can be done? An important principle of nutrition is that minerals and vitamins are very different beasts. Minerals are inorganic compounds. They cannot be synthesized from other molecules and must be obtained from the environment. To make plants rich sources of minerals, those plants must be persuaded to remove minerals from the soil and stockpile them.[5] In contrast, vitamins are organic molecules that can be synthesized from basic organic compounds like sugars and amino acids, given appropriate enzymes. To make plants rich sources of vitamins, those plants must be endowed with the ability to synthesize them.[4] The key is to take the part of the plant that is eaten (for cereals this would be endosperm of the seed) and modify it to increase its ability to store minerals and capacity to synthesize vitamins.

The idea of metabolically engineering plants to produce high levels of vitamins is not new. Many research papers have been published that describe plants with sometimes astonishing levels of key nutrients, and there have been several widely publicized successes such as Golden Rice, containing such high levels of the vitamin A precursor ß-carotene that rice grains appear golden yellow in color.[6] Although these successes have advanced the field significantly and provided hope that individual deficiency diseases can be eliminated or reduced, the enhancement of single nutrients still leaves a massive gap in the nutritional welfare of populations targeted with such crops. To avoid the disappointing yet inevitable outcome of such strategies, which could be to solve one deficiency problem only for another to arise in its absence, the focus for metabolic engineering strategies of the future should be to provide nutritionally complete crops.

Nutritional completeness means that a single staple crop, such as rice or corn, would provide every single micronutrient required by the human body and at appropriate levels such that the recommended daily intake (RDI) of all micronutrients would be achieved with the typical daily consumption of grain. Given our biological complexity, it is perhaps surprising that our bodies require such a small number of preformed organic molecules: a handful of amino acids, a couple of long-chain fatty acids, and a few vitamins. We also require a total of 16 minerals, but 11 of them are required in such small amounts that deficiency is almost unheard of, leaving only five—iron, zinc, selenium, iodine, and calcium—that need to be considered in nutritional enhancement strategies. The future of metabolic engineering should be looking at ways to achieve adequate synthesis of all essential compounds in a single crop, which means tackling multiple metabolic pathways at the same time.

Based on a recently developed combinatorial gene transfer system,[7] we were able to enhance three vitamins—ascorbate (vitamin C), folate (vitamin B_9) and ß-carotene (provitamin A)—in the endosperm of corn.[8] This was achieved by expressing genes for the necessary enzymes—phytoene synthase and carotene desaturase for provitamin A, dehydroascorbate reductase for ascorbate, and GTP cyclohydrolase for folate—each under the control of a promoter sequence that ensured the genes were expressed solely in endosperm tissue. The seed kernels of the resulting corn plants contained 169-fold the normal amount of ß-carotene, 6-fold the normal amount of ascorbate, and double the normal amount of folate, which means that a single typical serving would contain the entire RDI of ß-carotene, about one fifth the RDI for ascorbate, and adequate amounts of folate. If deployed, such a crop would simultaneously address three major nutrient deficiency diseases that are prevalent in the developing world without the need for supplementation or sourcing more exotic and expensive vegetables.

This is only the first step. We are already investigating the possibility of stacking even more genes in transgenic corn plants, causing the endosperm tissue to produce several other vitamins and essential amino acids, as well as encouraging it to accumulate zinc and iron from the environment.

Critics argue that more should be done to diversify the diet of the world's poorest people, but such criticism falls on deaf ears when there is no diversity to be found or it is out of the reach of impoverished people in the developing world. Nutritionally complete staple crops will provide an important short-term solution to the growing problem of global malnutrition, improving the health and wealth of subsistence farmers and in time allowing them to seek more conventional nutritional diversity.[9] We hope that by aspiring to produce nutritionally complete cereal crops, we can provide a workable solution to a major global health problem and can provide it in years rather than decades or centuries.

Acknowledgements: This work was supported by the Ministry of Science and Innovation (MICINN), Spain, (BFU2007-61413) and an ERC Advanced Grant (BIOFORCE) to PC.

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