SEEDS OF HEALTH

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WELCOME TO *SEEDS OF HEALTH*:

Welcome to the first issue of *Seeds of Health*, a newsletter to be published three times a year by the International Food Policy Research Institute, presenting recent scientific findings and issues relevant to agricultural strategies, and in particular plant breeding, for improving micronutrient nutrition in developing countries.

The motivation behind pursuing plant breeding as a means for addressing the serious problem of mineral and vitamin deficiencies in developing countries is that this strategy holds great potential for substantially improving human nutrition at low cost, while at the same time increasing agricultural productivity in an environmentally beneficial way (for more details see article on page 5).

The purpose of this newsletter is to provide information about ongoing research activities and recent scientific findings related to agricultural strategies for improved human nutrition, and to provide a forum for discussing issues on how best to pursue this strategy. The target audiences are (i) the multi-disciplinary group of scientists working on research activities related, directly and indirectly, to the plant breeding strategy, (ii) practitioners working for organizations already involved in the international effort to reduce micronutrient malnutrition, and (iii) the public who would like to become informed about this exciting mix of basic and applied research.

As a result of the rapid increases in staple food production in developing countries over the last three decades associated with the Green Revolution, staple food prices adjusted for inflation have fallen substantially. This has led to significant improvements in food security for the poor in terms of energy needs. However, the poor still cannot afford to buy sufficient quantities of vitamin- and mineral-rich non-staple foods such as fruits, pulses, and animal and fish products. The job of ensuring food security for the poor is only half-finished.

It is only in the last 15 years that human nutritionists have realized the enormous public health problem of micronutrient deficiencies, such as for iron, vitamin A, iodine, and zinc, whose underlying cause is poor dietary quality. Women and children are particularly vulnerable because of their greater physiological requirements due to reproduction and growth.

For plant breeding to become a viable, complement to presently implemented interventions such as supplementation and fortification, research must find satisfactory answers to a set of five fundamental questions:

1. Is it scientifically possible to breed for staple food crops whose seeds are micronutrient dense? Is there genetic variation in the characteristics that determine the density of micronutrients in seeds?
2. Can farmers be induced to grow nutritionally-enriched varieties? Are there agronomic advantages or disadvantages to mineral and vitamin dense seeds?
3. Will consumer or processing characteristics be altered? If so, can consumers be educated or otherwise induced to buy nutrient-dense varieties?
4. Will micronutrient intakes be increased to a significant degree? Will the extra nutrients be bioavailable (utilized)?
5. What is the relative cost-effectiveness of plant breeding as compared with other types of interventions to reduce micronutrient malnutrition?

Research and discussion relevant to this multi-disciplinary set of questions will be presented in this and future issues of *Seeds of Health*. Specific research activities discussed may not be explicitly integrated into an ongoing plant breeding program, but information provided will be relevant for guiding the direction of plant breeding efforts.

Howarth Bouis, Editor

GOLDEN RICE: PROOF OF CONCEPT AND BEYOND

Rice is the major staple food for hundreds of millions of people. It is generally consumed in its milled form with outer layers removed. The main reason for milling is to remove the oil-rich aleurone layer, which turns rancid upon storage, especially in tropical and subtropical areas. As a result, the edible part of rice grains consists of the endosperm, filled with starch granules and protein bodies, but it lacks several essential nutrients for the maintenance of health, such as carotenoids exhibiting provitamin A activity. Thus, reliance on rice as a primary food staple contributes to vitamin A deficiency, a serious public health problem in at least 26 countries including highly populated areas of Asia, Africa and Latin America.

A complementary intervention to existing strategies for reducing vitamin A deficiencies in the highest-risk countries is to fortify the major staple food, rice, with provitamin A through plant breeding. This can only be achieved by recombinant technologies rather than conventional breeding, due to the lack of any rice cultivars producing this provitamin in the endosperm. Both because the transformation of rice is well established and because the entire carotenoid biosynthetic pathway has been molecularly identified recently, it appeared feasible to introduce the complete provitamin A (ß-carotene) biosynthetic pathway into rice endosperm by genetic engineering.

RECENT RESEARCH AND NEW LINES

As reported earlier, three genes were added simultaneously to rice: *psy* and *lyc* (both cloned from Narcissus pseudonarcissus, and *cryt1* (cloned from Erwinia uredovora). The measured level of β-carotene in one gram of the transformed rice, commonly referred to as "Golden Rice," was 1.6 µg.

More recently we have been investigating an unexpected carotenoid pattern in the transgenic rice seeds. Currently it cannot be ruled out that the transformation using the bacterial crtI-gene promotes a hitherto unknown feedback mechanism enabling the transcriptional activation of carotenogenic genes. To test this, the chemical compound CPTA was administered to daffodil flowers, which turned reddish within 8 hours due to lycopene accumulation. However, concomitantly the carotenoid content was increased 2-3 times over the untreated controls. Northern blots revealed an increase of mRNA abundance for several carotenoid biosynthetic enzymes. This result cannot be explained by the well-known action of CPTA as a lycopene cyclase inhibitor, but indicates the presence of a novel regulatory mechanism.

One implication of this finding is that a construct containing only *psy* and *crtI* might be sufficient to install the entire pathway. Accordingly, reconstructed new single lines have been produced recently showing again yellow color. Carotenoid quantification showed again in the best performing segregating F0 line a carotenoid content of 1.6 µg per gram dry rice endosperm.

Work now in progress aims at increasing the provitamin A amount by first, identifying the metabolic rate-limiting "bottlenecks" in Golden Rice. New transformations are underway employing different endosperm-specific promoters, a codon-optimized crtI-gene and early pathway genes of the so-called non-mevalonate pathway of isoprenoid biosynthesis. One further approach aims at unifying high-iron rice lines with provitamin A lines since it is known that provitamin A is capable of increasing the bioavailability of iron.

Further proof-of-concept work aiming at measuring and enhancing the bioavailability and bioefficacy of the provitamin A are underway. Golden Rice is not expected to provide 100% of vitamin A in the diet, but to add to present intakes to reach vitamin A sufficiency. The current lines are only prototypes and efforts are underway at minimum to triple the amount of the provitamin in the endosperm. Certainly, a high priority for research is an evaluation of the bioavailability and bioefficacy and the pro-vitamin A contained in Golden Rice. This research has been hampered in the past by the need to produce a sufficient quantity of grain (multiple kilograms) for feeding trials in accepted model systems (pig, pre-ruminant calves, ferrets) in safety greenhouses in Europe and restrictions prohibiting field trials outside of greenhouses. However, novel analytical methods have become available (utilizing HPLC-linked electrochemical detection or deuterium labeling in combination with HPLC and mass-spectrometry) to significantly lower the amounts of rice required. Efforts are currently underway to allow import of Golden Rice into the U.S. where bioavailability investigations using these techniques can be conducted.

LEGAL SITUATION AND FIRST STEPS FOR EVENTUAL DISSEMINATION

The development of "Golden Rice" has been made possible by sequential funding (3 years each) by two agencies, first the Rockefeller Foundation and then a research program of the European Community (EC). While funding from the Rockefeller Foundation was free of obligations, EC funding required the participation of an industrial partner which would hold rights to inventions developed during the research. In this case, the industrial partner was Zeneca (merged recently with Novartis to form

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EDITORIAL GUIDELINES

This newsletter provides a forum for discussion on topics linking agriculture and human nutrition. Articles no longer than 1500 words and comments may be submitted to the editor for consideration for publication in subsequent issues. Research results reported are considered preliminary (unless otherwise stated by the author) and are not peer-reviewed. References to published materials are omitted for the most part, but may be obtained from individual contributors. Those who wish to receive hard copies or electronic files of future editions of Seeds of Health, please contact the editor.

Nutritional Genomics: Using Molecular Biology to Improve Human Health

Humans require a diverse, well balanced diet containing a complex mixture of both macronutrients and micronutrients in order to maintain optimal health. Macronutrients (carbohydrates, lipids, proteins and amino acids) make up the bulk of foodstuffs and are utilized primarily as an energy supply. Micronutrients are organic or inorganic compounds present in small amounts that are not utilized for energy, but are nonetheless needed for good health. Seventeen minerals and thirteen vitamins are deemed essential micronutrients in the human diet and are required at minimum levels to alleviate nutritional disorders.

From a nutritional perspective, humans have co-evolved with plants as their primary food source and many of the phytochemicals in our diet have significant consequences for human health. Indeed, these phytochemicals are thought to be a major reason for diets rich in plant foods being associated with lower morbidity and mortality in adult life.

Nutritional Genomics: An Approach for Dissecting and Manipulating Essential Micronutrient Pathways in Plants

The large number of ongoing sequencing projects in a variety of organisms represents one of the most significant developments for researchers in plant metabolism during the past two decades. Analysis of the growing DNA database shows a significant degree of inter-kingdom homology at the level of primary protein sequence. That many of these inter-kingdom orthologs are involved in basic cellular functions (e.g. protein synthesis, cell division, primary carbon and nitrogen metabolism) attests to the evolutionary conservation of these processes.

Nutritional Genomics is a general approach to gene discovery that is most applicable to compounds of nutritional importance that are synthesized or accumulated by plants and other organisms (e.g. vitamins and minerals). Nutritional Genomics takes advantage of the concept of metabolic unity among organisms through evolution and utilizes databases and in silico computer searches to rapidly move experimentally between organisms while remaining focused on the single pathway or enzymatic reaction of interest from the target organism. The approach is broadly applicable and allows one to take advantage of a variety of model systems having specific attributes that may be lacking or underdeveloped in the target organism (e.g. fully sequenced genomes, operons, pathway mutations, targeted gene disruptions or functional complementation).

While all plants have the enzymes necessary to synthesize or accumulate nearly all essential vitamins and minerals (with the exception of vitamin B12), staple foods often lack sufficient concentrations of many to meet RDAs. Thus, identifying the genes needed to enhance the levels of specific micronutrients in staple crops is an immediate goal that would have a significant impact on human nutrition worldwide. For example, because all plants synthesize vitamins, genes for their synthesis can be isolated and transferred from any plant system, including those being developed as genomic resources. Furthermore, these same vitamins are also produced in non-plant systems, such as bacteria and yeast, many of whose genomes have been fully sequenced. As such, prior biochemical, genetic and molecular data for vitamin synthesis in non-plant systems can be readily accessed by genomics and utilized to identify putative vitamin biosynthetic genes in plant databases. Once identified, they can rapidly be functionally tested by expression in the bacterial mutants used to elucidate vitamin synthesis in these organisms.

The Nutritional Genomics approach has recently been applied to the vitamin E and provitamin A biosynthetic pathways in plants. This work demonstrates the power of applying genomics to dissect vitamin biosynthesis in plants and one strategy for the targeted modification of plant vitamin content. Genomics will clearly accelerate isolation of biosynthetic genes for other plant vitamin pathways in the coming years. The use of these genes to manipulate plant nutritional content heralds an exciting new era for plant biologists and human nutritionists.

Molecular Genetic Approaches to Dissecting Plant Secondary Metabolism

For many secondary metabolites, including those of nutritional importance to humans, gene identification by classical biochemical approaches has given way to molecular genetic approaches. For example, Arabidopsis mutants exhibiting altered production of carotenoids, flavonoids, tocopherols and ascorbic acid have been used to establish the genetic basis for their synthesis. The increasing ease of utilizing expression in heterologous organisms has allowed functional cloning or characterization of steps in the synthesis of several vitamins and phytochemicals. The most successful has been the carotenoid pathway in which most of the biosynthetic enzymes have been cloned by color complementation in E. coli. More recently, plant enzymes involved in iron uptake and biotin, thiamin and vitamin E synthesis have also been cloned or functionally characterized using heterologous expression systems.

While Nutritional Genomics is a powerful approach for the limited number of plant compounds that are also synthesized in other organisms (e.g. vitamins), applying this approach to phytochemical pathways presents problems and limitations not encountered for vitamins. The extreme biochemical diversity and limited evolutionary distribution of many phytochemicals makes gene identification much more difficult. Orthologs for most phytochemical biosynthetic genes will not be expected outside the plant kingdom, which limits the usefulness of non-plant databases. Similarly, plants being developed as genomic resources (Arabidopsis, maize, rice) do not synthesize many of

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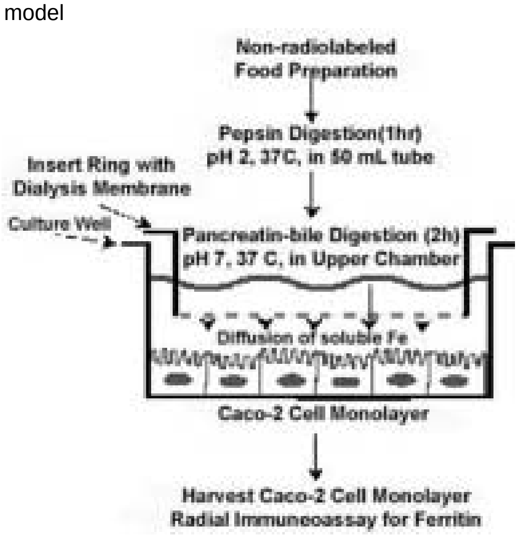
USING SIMULATED DIGESTION AND CELL CULTURE TO DETERMINE IRON BIOAVAILABILITY

In the past, efforts to understand the effects of various foods, food ingredients and processing on iron bioavailability have been hampered due to the high cost of performing animal and human feeding trials; thus, there is a great need for a rapid and inexpensive method for determining Fe bioavailability from various staple food crops as well as from different varieties of the same food crop in the presence of various meal components. The development of an in vitro model system that mimics the gastric and intestinal digestion of humans, coupled with culture of human intestinal epithelial cells (Caco-2) shows great promise in satisfying this need.

In this model system, foods undergo simulated peptic digestion followed by intestinal digestion in the presence of Caco-2 monolayers (Figure 1). This system provides a measurement of iron uptake by living cells, in addition to measurement of the solubility of the iron. The system is unique in that it allows uptake to occur simultaneously with food digestion under the normal physiological pH conditions associated with the absorptive surface of the intestinal tract.

Formation of ferritin, an iron storage protein, is a known response to iron uptake and is used as an indicator of iron availability. Ferritin formation is easily measured via ELISA or radioimmunoassay. The use of ferritin formation makes this model system extremely useful, eliminating the cost and controversy associated with food radiolabeling and enables foods to be assessed for iron availability directly from the producer or food manufacturer. When used as a prelude or in conjunction with human trials, it can address questions not feasible or cost-effective to study in vivo. This model system has the potential to refine experimental objectives prior to performing the more expensive human trial, thus improving the effectiveness of research funding.

Figure 1: Diagram of in vitro digestion/Caco-2 cell culture model



Published results by this author and coworkers have demonstrated that this model system is qualitatively similar to human studies under a variety of food or meal conditions. For example, they have documented the promotional effects of meat (muscle tissue) and ascorbic acid on Fe uptake in this system. They have demonstrated that phytic acid and tannins are strong inhibitors of iron uptake. They have shown that heme iron is less affected by phytic acid than nonheme iron. Also they have shown that specific forms of Fe such as Fe-ascorbate, Fe-citrate, Fe-EDTA, elemental Fe and Fe-sulfate exhibit qualitatively similar availability in this system as in human studies under similar conditions. It should be noted that direct simultaneous comparisons of this model with human studies have not yet been conducted, only comparisons with published effects found in the literature.

The in vitro digestion/Caco-2 cell model system has numerous applications and potential for determination of iron availability. It is an obvious tool for food manufacturing companies to study effects of formulation and processing on iron availability. Examples of improved products would include fortified breakfast cereals, infant formula, human milk fortifiers for preterm infants and pharmaceutical iron supplements. The end result of this research will be food products of increased nutritional quality and marketing advantage for the producer.

Another important application of this model is the potential to combine different ratios of plant and animal foods to determine the proportions that optimize trace element bioavailability. This approach can be used to formulate optimal food patterns using local and or traditional foods for populations worldwide.

Another useful application of this model system is for development of improved staple plant foods that provide more bioavailable micronutrients. Literally hundreds if not thousands of different genotypes exist for staple crops such as maize, wheat, beans and rice. Plant breeders can assess traits of plants for nutrient content but have been lacking a means of correlating genetics with nutrient availability. Development of plant foods with increased density of micronutrients and nutritional quality is believed to be a sustainable approach to alleviating the "hidden hunger" of micronutrients such as iron, observed at alarming incidence in developing countries. Populations in these areas have a very limited diet, often subsisting on a single staple food crop such as rice encompassing the bulk of their diet. Developing staple plant foods with improved micronutrient content and availability is seen as a means to improve the lives of millions of people. An example of application of the in vitro digestion/Caco-2 system to identify promising genotypes of staple food crops is shown in Figure 2. In these experiments 15 Fe-dense genotypes of unpolished rice were compared to a rice known as Nishiki. It is interesting to note that the genotypes exhibiting low Fe availability were noticeably higher in tannin content. Fe availability was not correlated with Fe content,

Using Simulated Digestion... continued on page 11

Plant Breeding: A Cost-Effective Approach for Reducing Micronutrient Malnutrition

Over 3 billion people in the world suffer from iron deficiency. Because of their elevated requirements, women of reproductive age and young children are at particularly high risk. It is estimated that 50% of pregnant and 40% of non-pregnant women in developing countries are anemic. Iron deficiencies during childhood and adolescence impair physical growth and mental development and learning capacity. In adults, iron deficiency reduces the capacity for physical labor.

It is only in the past fifteen years that nutritionists working in poor countries have recognized the enormity, for which new and compelling scientific evidence is rapidly accumulating, of the problem of mineral and vitamin deficiencies.

A World Bank publication estimates that deficiencies in iron, vitamin A, and iodine alone, at levels of malnutrition that presently exist in South Asia, cause economic losses equal to 5% of GNP each year due to sickness, poor work performance, lost education, and other factors.

Initially there was some optimism that supplementation and fortification programs, which treat the symptoms but not the underlying cause of micronutrient malnutrition (which is diets of poor quality, consisting primarily of staple foods to meet basic energy requirements), could solve much of the micronutrient deficiency problem quickly and easily. In some situations the payoff to investments in these programs has been high. However, in general these programs have not worked as well as in developed countries because of poor institutional infrastructure, among other reasons.

Plants which fortify themselves is an inherently appealing approach in that they provide a delivery system for nutritional supplements that would have few recurring costs. Once farmers have the appropriate seeds, they could continue to grow them year after year. In contrast, supplementation or fortification programs require ongoing expenditures to keep delivering required nutrients. Furthermore, if the nutrient dense crops are unchanged in appearance, processing and cooking qualities, and taste, they could provide higher levels of micronutrients in the diet without requiring any apparent dietary change.

Why Has this Strategy Not Been Tried Before?

The few past attempts at breeding for nutritional characteristics (usually for vitamins or protein) have encountered substantial barriers to producing both nutrient-dense and high-yielding (and so high-profit) varieties which would be accepted by farmers. Thus, the usual presumption has been that giving plant breeders an additional characteristic (better nutritional quality) will be too costly in terms of the opportunity foregone in developing higher-yielding genotypes. However, as science makes new advances, it is often the case that conventional wisdom requires rethinking. New knowledge in three areas has led to a reevaluation.

First, as already alluded to above, the payoff to improved micronutrient content is much higher than previously thought; and the difficulties are greater than previously thought of treating the micronutrient problem using methods which have worked in developed countries. Second, advances in plant breeding techniques can speed the process and so lower the costs of breeding.

Third, and perhaps most importantly, relatively recent research shows that nutritional balance - specifically for trace minerals - is just as important for plant nutrition as for human nutrition. Breeding for trace mineral dense seeds improves plant nutrition, reduces input costs, and improves yields and profits on trace mineral deficient soils. Agricultural productivity in poor countries may be substantially improved, at the same time as the nutritional quality of seeds for human consumption is improved.

Agronomic Advantages

The basic reasons for these agronomic advantages may be stated in a simple way. Plant nutrition may suffer from trace mineral deficiencies in a number of ways (for example, zinc and manganese play key roles in preventing root disease in wheat). These "deficiencies" are caused not by the physical absence of trace minerals in the soil (in fact, sufficient amounts are usually available for hundreds or thousands of crops - soils may never be depleted due to additions such as through windblown dust), but by the fact that the trace minerals are bound chemically to other elements that make them "unavailable" to plants. Such soil "deficiencies" are widespread in developing countries.

Certain plant genotypes, however, are more efficient than others in the uptake of trace minerals from soils (for example, their roots exude substances that chemically "unbind" minerals in the soil, resulting in their becoming available to plants). Plant breeding may select for such "efficiency" characteristics, including the characteristic of translocating high amounts of trace minerals to the plant seeds. When replanted in "deficient" soils, such mineral-dense seeds have been shown to be more vigorous and disease-resistant, which, in turn, leads to higher plant yields.

Comparative Costs

What are the relative costs of plant breeding as compared with supplementation and fortification? Vitamin A supplements cost 25 cents per tablet (inclusive of administrative and distribution costs) and must be administered twice a year. If only 1 in 12.5 persons out the approximately 1.25 billion people in South Asia were to receive supplements (100 million people treated), the

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CGIAR Micronutrients Project Update

MICRONUTRIENT VARIABILITY IN RICE

Since 1992, researchers at IRRI have been evaluating the genetic variability of Fe concentration in rice grain. In 1995, the research was expanded to include Zn. The range in Fe and Zn concentrations in brown rice within the eight sets of genotypes (n=1,138) tested in one study was 6.3 µg g-1 to 24.4 µg g-1 for Fe and 13.5 µg g-1 to 58.4 µg g-1 for Zn. (See table 1) Thus, within those genotypes tested, there was about a fourfold difference in Fe and Zn concentrations, suggesting some genetic potential to increase the concentrations of these micronutrients.

The highest grain-Fe concentrations (i.e., ranging from about 18 µg g-1 to 22 µg g-1) were found in several aromatic rice varieties such as Jalmagna, Zuchem, and Xua Bue Nuo. These same aromatic lines also contained the highest grain-Zn concentrations (ranging from about 24 µg g-1 to 35 µg g-1). Further research using F2-derived populations demonstrated that the aromatic trait was not pleiotropic for grain-Fe or grain-Zn concentrations and, therefore, this trait may be used to screen for high Fe and Zn levels in rice grain, but the linkage is broken at a low frequency.

Several studies were carried out at IRRI to examine the effect of soil and climate on grain-Fe and grain-Zn concentrations among genotypes. Factors studied included wet season versus dry season, normal versus saline soils, acid versus neutral soils, and N supply. The data from these various studies demonstrated that high-Fe and high-Zn grain traits are expressed in all rice environments tested although there is some evidence of significant genotype x environment interactions that can ultimately affect Fe and Zn concentrations in extreme environments.

A high-iron trait can be combined with high yielding traits. This was demonstrated in the serendipitous discovery of an aromatic variety already in the IRRI testing program -- a cross of a high yielding variety (IR72) and a tall, traditional variety (Zawa Bonday) from India, from which IRRI identified an improved line (IR68144-3B-2-2-3) with high a concentration of grain iron, about 21 ppm in brown rice. This elite line has good tolerance to rice tungro virus and has excellent grain qualities. Yields are about 10% below IR72 but in partial compensation, maturity is earlier. This variety has good tolerance to mineral deficient soils such as P, Zn, Fe. It has no seed dormancy and excellent seedling vigor, suggesting that it could be a good direct-seeded rice. Among the high-iron varieties were several aromatic rice genotypes, an observation which led to the discovery of IR68144-3B-2-2-3 . Aromatic rice was consistently higher in grain iron concentration and often also in zinc than its non-aromatic counterparts.

A comparison of Fe content at different polishing times for high-iron traditional varieties (red pericarp) with IR64 and IR68144-3B-2-2-3 (white pericarp) was undertaken which demonstrated a strong interaction between genotype and time of milling. Grain color appeared to be associated with the amount of iron content. The grain appearance of red pericarp varieties like Jalmagna, and Tong Lang Mo Mi became fairer as polishing time increased. Changes of color were observed in Jalmagna and Tong Lang Mo Mi from 15-45 minutes polishing time, corresponding to large declines in Fe content.

For IR64, a popular commercial variety with the lowest Fe in brown rice, the Fe content dropped by more than one-third with 15 minutes polishing (the time equivalent to that of commercial polishing); after 15 minutes Fe content remained almost unchanged. A loss in Fe content of about one-third at 15 minutes milling was observed for two high-iron traditional rices, Jalmagna and Tong Lang Mo Mi, but their iron concentrations continued to decrease substantially as polishing time increased. These observations suggest that much of the iron is in the outer layers of the grain. However, Xua Bue Nuo, a traditional variety from China, and high-iron IR68144-3B-2-2-3 were less affected by polishing time. At 15 minutes polishing, IR68144-3B-2-2-3 had about 80% more iron than IR64.

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Table 1. Brown rice Fe and Zn content of rice varieties grown under similar growing conditions in six different sets

Variety set	No. of samples	Fe (mg/Kg) Mean ± SE (Range)	Zn (mg/Kg) Mean ± SE (Range)
Traditional & improved varieties	140	13.2 ± 2.9 (7.8 - 24.4)	24.2 ± 4.6 (13.5 - 41.6)
IR breeding lines	350	10.7 ± 1.6 (7.5 - 16.8)	25.0 ± 7.6 (15.9 - 58.4)
Tropical japonicas	250	12.9 ± 1.5 (8.7 - 23.9)	26.3 ± 3.8 (15.0 - 40.1)
Popular varieties and donors	199	13.0 ± 2.5 (7.7 - 19.2)	25.7 ±4.6 (15.3 - 37.3)
Promising lines (NCT)	83	8.8 ± 1.3 (6.3 - 14.5)	25.4 ± 4.2 (17.0 - 38.0)
New plant types	44	16.7 ± 2.1 (11.5 - 24)	29.6 ± 3.2 (23 - 36)
Wild rice & derivatives	21	15.6 ± 2.3 (11.8 - 21)	37.9 ± 8.6 (23 - 52)
Aromatic rices	51	14.6 ± 3.2 (10.8 - 23.2)	31.9 ± 6.0 (23 - 50)

Research on Trace Minerals in Common Bean

Food legumes in general contain appreciable quantities of iron and other minerals. Although legumes are often cited as a complement to cereals in terms of amino acid content, they also make a particularly important contribution to micronutrient nutrition. Decreasing legume per capita consumption in India is considered to be one possible cause of increasing iron deficiency, illustrating the importance of legumes in the diet. The common bean (Phaseolus vulgaris L.) is the most important grain legume for direct human consumption, being especially important in Eastern Africa and Latin America. CIAT's objective in participating in the CGIAR Micronutrients Project has been to assess the feasibility of improving common beans for micronutrient content, especially iron and zinc.

Genetics of mineral content

Four populations had been prepared as recombinant inbred lines (RIL) for the development of molecular markers, three of which have been analyzed. Two of these populations were derived from crosses of Mesoamerican parents, and one population from a cross of Andean parents. Thus the two major gene pools of common bean were represented. The recombinant lines reveal aspects of the genetics of iron and zinc content in the parental materials. Results of all three populations are similar, and only the results of one population are presented graphically.

In all three populations both iron and zinc content in the RIL presented a continuous distribution, which is to say, mineral content behaves as a quantitative trait. The parental accessions were in each case very close to the extremes of the populations, and very few progeny exceeded the values of the parental genotypes. This suggests that almost all of the favorable alleles came from the high iron parent. The only exception to this rule was the population of G11350 x G11360, a Mesoamerican cross, in which several progeny either inferior or superior to the parents were observed in iron content. The number of segregating lines that presented iron contents similar to the high parent suggest that the number of genes involved could be in the range of 4 to 7. These results are being confirmed with a study of the quantitative trait loci (QTLs) that control micronutrient accumulation in beans. We hope to focus on certain parts of the bean genome to determine if desirable alleles for higher mineral content are located at the same loci in different populations developed specifically for this purpose. We also plan to integrate the information about the genetic locations of QTLs for micronutrients with those for other agronomic traits so that we can select for the best recombinants from crosses between high micronutrient lines and the elite varieties.

Breeding Using Advanced backcrossing

An advanced backcross breeding procedure has been used to transfer high micronutrients from a wild accession from Mexico (G10022) to three recurrent parents, including the Andean variety ICA Cerinza and two Mesoamerican varieties, DOR390 or Tacana, and Pinto Villa. For the advanced backcross population with Cerinza, iron and zinc content was normally distributed, indicating again that micronutrient accumulation is probably a quantitative trait. The range in mineral content was 55 to 103 ppm for iron and 26 to 43 ppm for zinc. The number of lines with significantly more iron and zinc than the recurrent parent, Cerinza, was 26 and 24, respectively, therefore it appears that transgressive segregation plays an important role in the accumulation of genes or QTLs for higher mineral content. The correlation between iron and zinc content in the lines was significant (r=0.373), indicating that the linked genes or the same genes may be involved pleiotropically in controlling the accumulation of both minerals. If the same QTLs contribute simultaneously to both iron and zinc content, it may be easy to select for these traits jointly. The advanced backcross method had been shown previously to be a useful method for incorporating wild germplasm into cultivar breeding programs. Although wild beans have been used before to transfer resistance to diseases and insects, this is the first study to breed higher nutritional quality from wild beans.

With this work we are close to producing advanced breeding lines in some classes with higher iron content. The advanced backcross method is especially promising for creating high iron lines that are commercially undistinguishable from their recurrent parent. However, the routine creation of high mineral varieties will require more efficient phenotypic screening procedures and the use of marker assisted selection.

Studies to date suggest that iron content of common bean could be increased by 60-80%, while potential gains in zinc content would be more modest, perhaps around 50%. Genetic differences have been expressed over environments and seasons, offering prospects that genotypes selected in one environment for high iron or zinc will express superior levels of minerals in other environments as well. Although the genetics of iron and zinc content appears to be complex, marker assisted selection could be used to pyramid genes for higher mineral content. Correlations among mineral contents suggest that the improvement of one mineral may simultaneously improve content of other minerals, thus multiplying the impact of the effort. With the prospect of creating almost finished varieties with high iron content, the stage will soon be set for testing of the health benefits from consuming these bean varieties in communities where iron deficiency anemia is prevalent.

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CGIAR Micronutrients Project Update

Breeding for Trace Minerals in Wheat

The objective of research under the CGIAR micronutrients project was to further our understanding of the possibility of enhancing the micronutrient content in the grain of wheat through plant breeding. In a breeding program before a new breeding objective is adopted, it is necessary first: 1) to establish the level of genetic diversity for that trait, 2) to identify how many genes are involved, and 3) to determine what is the heritability of the trait. Our research has focused mainly on the first component. In addition, we have been studying the effect of two important genes in the Centro Internacional de Mejoramiento de Maiz y Trigo's (CIMMYT) wheat germplasm that could potentially have an effect on micronutrient concentration in the grain. These are the rht genes that confer the dwarf character to CIMMYT germplasm and the 1B/1R translocation, which is the transfer of a chromosome segment from rye into wheat. Historical changes in Fe and Zn concentration in the grain of wheat of CIMMYT releases between 1950 and 1992 were also part of our study.

In our search for genetic material with high levels of Fe and Zn concentration in the grain of wheat, we have identified the following sources in order of importance; wild relatives of wheat, landraces, bread wheat, triticales and durum wheat. There remains great potential to find even better genetic material given that less than 1% of the germplasm available at CIMMYT has been screened. A significant positive correlation has been found between Fe and Zn concentration in the grain of wheat, suggesting that these two traits may be combined relatively easily during breeding.

The production of semidwarf wheat through the introduction of the rht genes has resulted in a yield increase in both bread wheat and durum wheat. However, this is associated with a reduction in Fe and Zn concentration in the grain of some bread wheat genotypes, but not in durum wheat. The presence of the 1B/1R translocation in CIMMYT's wheat germplasm perhaps has had a positive effect on the concentration of Fe and Zn in the grain, but a negative effect was ruled out.

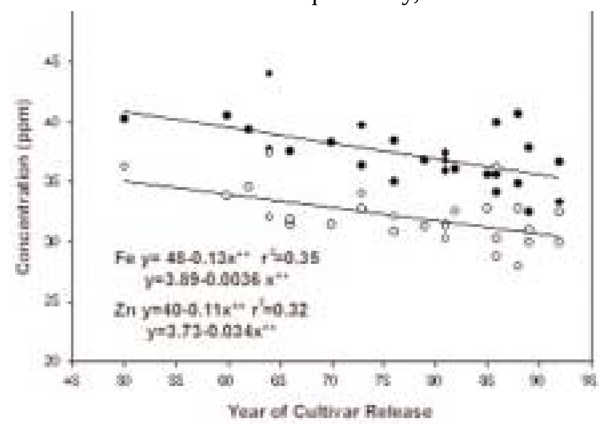
Green Revolution Varieties of Wheat

A historical set of bread wheat cultivars, which represent progress in the CIMMYT bread wheat breeding program from 1950 to 1992 were studied to determine if changes in Fe and Zn concentrations had occurred over time. The first two released varieties included in the study were Yaqui 50 and Nainari 60, which are traditional tall varieties. The remaining entries were semi-dwarf wheat representing progress in the period of semi-dwarf improvement at CIMMYT.

As expected, the study found a strong linear relationship between the year of release and grain yield. Seventy-two percent of the variability in grain yield between 1950 and 1992 was explained by the year of release. In absolute terms the estimated rate of progress in grain yield has been 51 kg/ha/year (a cumulative total of about 2.1 tons/ha over 42 years from a base of about 4 tons/ha) and in relative terms 1% per year. This rate of yield gain is comparable to that of CIMMYT breeding work elsewhere in the world.

Regression analysis indicated a small by statistically significant negative trend in Fe and Zn concentrations. Variation around this trend was substantial (see Figure 1); only 35% and 32% of the variability in Fe and Zn concentration, respectively, were explained by year of release. Several varieties released by CIMMYT throughout 1960-1992 had concentrations of Fe and Zn comparable to those found in first two tall varieties included in the study, while concentrations in other releases were substantially lower. In absolute terms the estimated reduction in Fe and Zn concentration has been 0.13 ppm/year (a cumulative total of about 5 ppm over 42 years from a base of 40 ppm and 35 ppm for Fe and Zn concentrations, respectively) and in relative terms 0.3% per year.

Figure 1. Fe and Zn concentration in the grain in a historical set of CIMMYT releases in the Yaqui Valley, Mexico.



Future Research

In future research, the very high values of Fe and Zn in the some wheat lines need to be confirmed in a trial where all the best material is planted in the same location and year. In addition, it is important to determine if these high levels of Fe and Zn in the grain can be maintained in high yielding material. We have already started to make crosses between bread wheat and some of the wild species to answer this question.

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CGIAR Micronutrients Project Update

Carotene in Cassava Roots and Leaves

Cassava has traditionally been regarded as the poorest of the staple foods in nutritional quality. This is probably because of the low protein content of its storage roots, the primary edible product. However, cassava has considerable nutritional potential because its leaves can be used as well, and they have high protein concentration. As both leaves and storage roots are used fresh, they also have the potential to supply both water soluble and fat-soluble vitamins. The ongoing objective of research under the CGIAR Micronutrients Project is to assess the amounts of micronutrients in cassava and the degree of genetic variability that could be exploited to improve the micronutrient density and balance of nutrients through breeding.

Cassava is an important staple food for 500 million people, and is outstandingly adapted to poor soils and environments. Cassava therefore will grow and can be relied on to produce food under conditions where other staples would fail. Cassava is tolerant of acid and alkaline soils, low fertility, pests and diseases and seasonal drought. High cyanide types are resistant to animals and the roots can be "stored" in the ground before harvesting for considerable time as a drought reserve. All these features together justify an examination of how its nutritional value could be harnessed and improved.

The cassava core collection (601 genotypes) was evaluated for root and leaf contents of micronutrient minerals, ascorbic acid, and carotene. Wide genetic variability was observed for all measurements, indicating that there is good potential for exploiting and improving the nutritive value of cassava. There seems to be little correlation between the levels of any micronutrient in roots and leaves.

Carotene concentrations

The inheritance of carotene concentration in cassava was studied in an F2 population of a white X yellow cross. Intensity of root color was found to be highly correlated with carotene concentration (see Table 1). However, the range of genotype variability in carotene content was quite high, with a range of 0.6-2.4 mg of carotenes per 100 grams of fresh root even for deep yellow and orange roots. In fact, the range of carotene concentration within any particular root color class (white, cream, yellow, deep yellow, orange) was sufficiently high that a quantitative evaluation of carotene of clones pre-selected according to root parenchyma color is justified. The inheritance of carotene concentration appears to be determined by two genes, one controlling the transport of the product of precursors to the roots, the other responsible for the accumulation process.

Stability of vitamin content after different processing procedures

The effects of different processing methods on carotene content were studied in a group of 28 genotypes. On average, boiling reduced carotene content the least (34%), followed by oven-dried flour with a 44% reduction. Sun dried flour reduced the carotene concentration to the lowest level (73% reduction). Although the correlation among different processing methods across genotypes was significant, genotype-specific effects were sufficiently strong that genotypes with the highest carotene concentration in the fresh root controls were not the ones with the highest concentration after processing. Although carotene levels declined significantly during processing, considerable amounts of carotenes remained, particularly when oven drying was carried out. Gari (a dry flour produced from cassava roots) obtained from yellow-root cultivars presented concentrations of carotene of up to 1.13 mg/100g.

The carotene content was considerably more stable than ascorbic acid after the different processing procedures evaluated. On average 50% of the original carotene content remained after boiling or drying the roots, whereas only 14% of the ascorbic acid was recovered after the same treatments.

Table 1.
Average carotene concentration in cassava roots classified according to root color

Root color	Numerical scale	Carotene (mg/100g)	Stand. dev
White	1	0.13	0.48
Cream	2	0.39	0.28
Yellow	3	0.58	0.28
Deep Yellow	4	0.85	0.17
Orange	5	1.26	0.11

It appears that cassava's reputation for poor nutritive value need not be so. First, there is substantial genetic variation that can be exploited to improve its micronutrient density, and probably its protein content as well. Second, the use of the leaves as a vegetable along with the roots as staple, as is practiced in Africa, can do much to balance the diet, especially for protein, micronutrients and calcium. Third, cassava, because of its high carotene germplasm, can deliver varieties with superior concentrations of iron, zinc and pro-vitamin A, to exploit the synergies that operate in absorption, internal transport and function between these three micronutrients. If so and as with maize but not wheat and rice, cassava is in a favorable position in that it has high carotene types to develop immediately. To do so requires the education of all sectors of the food system to the advantage of yellow staple over white.

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cost would $50 million per year or $500 million over a decade. Iron fortification costs about 10 cents per person per year. Reaching 40% of the population (500 million people some of whom are iron-deficient) with an iron-fortified food would also cost $50 million per year, or $500 million over a decade. Recurrent costs of supplementation and fortification remain constant year after year.

If successful, plant breeding has tremendous leveraging power in that research output at central facilities (a one-time cost except for maintenance breeding) eventually may be adapted by national programs to growing conditions all over the world. For example, a CGIAR-wide proposal on "biofortification" is proposing to spend $12.5 million over a decade per crop, or $25 million for rice and wheat the major staple foods for South Asia. This amount includes costs of developing nutrient-dense germplasm, nutritional testing, some adaptive breeding, dissemination costs for limited areas, and impact studies.

The additional profits made by farmers could well justify the research investment. A CIMMYT wheat breeder based in Turkey, where soils are particularly zinc-deficient, has estimated that, if the zinc-dense seed varieties already available on a commercial basis in Australia were adapted to growing conditions in Turkey, Turkish wheat farmers would save $75 million annually in reduced seeding rates alone (seeding rates could be reduced from an average of 250 to 150 kilograms per hectare on 5 million hectares; a ton of wheat might sell for about US$150 on the world market). This does not even count the benefit of higher yields.

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Golden Rice continued...

Syngenta). This EC funding obligation has impacted the current legal status of the Golden Rice project, which continues on two tracks, one being non-commercial (or "humanitarian"), the other commercial.

Syngenta received through the involvement of the German start-up-company Greenovations, an exclusive license for the commercial use of the technology in developed nations. Simultaneously Syngenta granted back to the inventors (Potrykus/Beyer) the exclusive license and the right to grant sub-licenses for its non-commercial use. Agreement has been reached between the two tracks which we believe serves our mutual interests. For example, all knowledge derived from research on the industrial marketing track will be made available free of charge to the humanitarian track of the project. Moreover, a severe intellectual property rights (IPR) problem in the humanitarian project that could not be dealt with by private persons nor by their Universities, has been solved thanks to the input of Dr. Adrian Dubock of Syngenta. Development of Golden Rice required the use of various technologies that are properties of several industrial companies, and some Universities. The non-commercial use of Golden Rice in the developing world required the written consent of the respective IPR-holders. The necessary multi-lateral negotiations required – as it turned out – inter-industrial interaction involving the respective expertise of various parties involved.

The humanitarian project has established an advisory panel, called the Humanitarian Board, which meets regularly. Syngenta is represented on the Board along with scientists of various disciplines, some working for international and other agencies involved with assistance programs to developing countries. The complementary expertise of the individuals involved ensures the flow of information between the two tracks, and ensures that all steps taken are in accordance with the intra-project legal requirements and with respect to the laws of various countries interested in receiving the technology. Via the humanitarian board, a non-commercial license can be obtained by national and international research institutes. It is at these institutes that further development, such as the transfer of the β-carotene trait into local varieties by classical breeding or by transformation, or the breeding of provitamin A varieties with stable and high yields, will be carried out.

In January 2001, the first transfer of the technology took place to the International Rice Research Institute (IRRI) based in the Philippines, a member institute of the Consultative Group on International Agricultural Research (CGIAR) with a long standing and proven expertise in breeding improved rice varieties for dissemination to developing countries, primarily in but not restricted to Asia. In addition, facilitated by the "Indo-Swiss Collaboration on Biotechnology (ISCB)," further research and development of Golden Rice in India is being pursued in collaboration with national research institutes. Dr. Hoa, a Vietnamese visiting scientist, has transformed several local varieties. She will take these seeds back to Vietnam to conduct further research there, in accordance with a sub-license agreement with the Qcuu Long Delta Rice Research Institute. A possible transfer to China is currently being discussed with the Chinese Ministry of Science and Technology.

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Nutritional Genomics continued...

the best-characterized health-promoting phytochemicals and will lack many target genes. For these and other reasons, researchers studying a specific phytochemical are often limited to a few plant species that synthesize and accumulate the target compound(s) to high levels (e.g. 0.5-5% of dry weight). The question becomes: How can researchers access and utilize this information resource?

One possible approach, not yet fully implemented, applies the technologies of large-scale DNA sequencing and DNA microarray expression studies to non-model plant species and their natural variants that accumulate high amounts of a target compound. This approach will facilitate the identification of a limited number of candidate genes pertinent to the pathway of interest for further study. Once identified, such genes (and orthologs) can be functionally analyzed by heterologous expression or knock out approaches in a variety of organisms including bacteria, yeast, maize and Arabidopsis.

Future Directions

Research to improve the nutritional quality of plants has historically been limited by a lack of basic knowledge of plant metabolism and the often daunting task of dissecting whole branches of plant secondary metabolism. The advent of genomics provides new integrative approaches to plant biochemistry that allow crossing of species, family and phyla barriers. As a result, the increase in our basic knowledge of plant secondary metabolism during the coming decade will be truly unparalleled and will place plant researchers in the position of being able to modify the nutritional content of major crops to improve aspects of human health. For essential minerals and vitamins that are limiting in world diets, the need and way forward is clear, and improvement strategies should be pushed forward. However, for many other health-promoting phytochemicals, decisions will need to be made regarding the precise compound(s) to target and which crops to modify such that the greatest nutritional impact and health benefit is achieved. Because these decisions will require an understanding of plant biochemistry, human physiology and food chemistry, strong interdisciplinary collaborations will be needed among plant scientists, human nutritionists and food scientists in order to ensure a safe and healthful food supply for the coming century.

Finally, while manipulation of micronutrient levels using the Nutritional Genomics approach is both agriculturally attractive and scientifically feasible, more traditional approaches exist and need be be pursued both independently and in conjunction with emerging technologies. In the short term, the early integration of emerging technologies with traditional approaches may be the most straightforward research program to implement for improving human nutritional status, especially in developing countries.

(excerpted from: DellaPenna, D. (1999) Nutritional Genomics: Manipulating Plant Micronutrients to Improve Human Health. Science 285: 375-379)

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Using Simulated Digestion...continued

which ranged from 15-40 ppm in these samples. These studies were performed at a fraction of the cost of in vivo trials and required only 3 months to conduct the in vitro digestion experiments. Similar trials are ongoing for genotypes of wheat and maize.

To summarize, it is already evident that this model is useful as a screening tool for general effects on iron availability to humans. It is critical at present that we compare this model directly with ongoing human trials to determine how closely this model can predict human iron absorption. If necessary, adaptations of the model can then be developed to refine the model system. The collective opinion of bioavailability experts worldwide is that simulated digestion coupled with Caco-2 cells can represent the major effects of foods on human Fe uptake. The question is just how good a system it can become.

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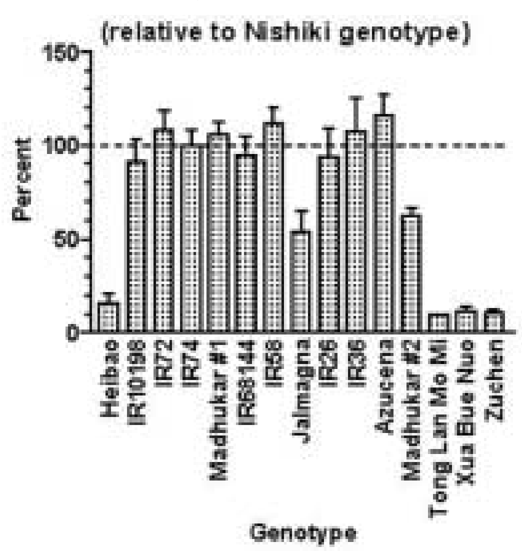
Figure 2: Comparison of iron availability from genotypes of unpolished (brown) rice. Caco-2 cell ferritin formation values from all varieties were compared to a common genotype (Nishiki) and expressed as a percent relative to that genotype. Values are mean ± SEM, n=5-6.



Needed: More Emphasis in Using Food-Based Strategies to Improve Human Nutrition

The great strides gained in increasing the supply of grain in the last 25 years attest to the undeniable importance of food based strategies in solving hunger and malnutrition. In order to achieve a greater and faster improvement of human nutrition, food based strategies which focus on commonly consumed foods are necessary.

The past focus on staple foods was well warranted. However, though important for contributing energy and nutrients, staple foods are not the only foods eaten. Small amounts of other foods such as vegetables, fish, pulses, fruits and spices are also consumed. These commonly consumed foods make up an essential part of the everyday diet and should play a much larger role in food based strategies and the setting of goals. Focus on these foods is opportune as the thrust on grains has detracted attention from them and their production has diminished. In Southeast Asia, as the area under rice has increased, pulses are now grown on a smaller area and their production has fallen significantly.

Increasing the access to these habitual foods for poor population groups is thus a goal that should be explicit in food based strategies. To achieve this, these foods first need to be identified and given the status and priority they deserve. In Bangladesh and in many Southeast Asian countries, small fish and other aquatic animals are an important part of the everyday diet of the poor and contribute considerably to the intakes of animal protein, iron, vitamin A, calcium and zinc. Recent studies have shown that calcium bioavailability from small fish eaten with bones is as high as that from milk. No inclusion of these foods in food based strategies has been made in spite of the fact that they are well liked, are relatively inexpensive, are frequently consumed, have high nutrient density and increase nutrient bioavailability. Efforts have been focused on increasing the production of large fish. This has not benefited the poor who eat small fish.

In some countries, wild foods such as vegetables, fruits and animals are commonly consumed, greatly contributing to vitamin and mineral intakes. Therefore, strategies that promote and protect common property areas are important to ensure the continued access of such wild foods to the poor. Also, knowledge of the importance of these foods in the local food culture and meal pattern, as well as their nutrient density, is necessary in order to set specific goals regarding which foods should be given greater priority and how the areas where they are grown should be protected and managed.

Strategies that target only foods eaten during meals and not also those eaten outside meals can limit the effect of food based strategies. In some parts of the world, the largest proportions of nutrients such as fat, vitamins A and C are supplied from foods eaten between meals and consumed out of the house. Furthermore, seasonality of food intake is a common feature of food intake in developing countries. This should also be considered in food based strategies. Greater production and consumption of high-yielding vitamin A-rich fruits can build up needed vitamin A body stores. Breeding of new varieties that can increase off-season production of such foods can offset seasonal reductions in nutrient intakes. In addition, nutrition education focused on promoting positive perceptions of foods in relation to health and nutrition, as well as beneficial local habits and customs must be made an integral, quantifiable goal in the strategies to be chosen.

Selecting and breeding edible plants and animals with higher nutrient density, and/or higher contents of enhancers and lower contents of inhibitors of mineral absorption offer further exciting avenues for setting specific goals for food based strategies. A three-fold increase in iron and zinc content in rice can increase intakes of these minerals immensely in poor populations eating rice, as they consume large quantities. Using traditional processing methods and new technologies to reduce phytic acid, a potent inhibitor of mineral bioavailability in cereals is also important.

In many developing countries, there are definite quantifiable targets for the annual supply of staple foods to be achieved at the national level. These targets are effectively used to direct strategies and set explicit goals with respect to productivity and other measures to ensure that the national supply is met. With respect to other commonly consumed foods, there are some general policy guidelines regarding increased overall production, but rarely are strategies and goals set. The World Summit for Children in 1990 set specific nutritional goals regarding quantifiable reductions in single nutrient (iron, iodine and vitamin A) deficiency within a specific time frame. This enabled the formulation and implementation of supplementation and fortification programmes with defined strategies and goals. In the two conferences which dealt with food, the International Conference on Nutrition in 1992 and the World Food Summit in 1996, no specific goals were set with respect to foods, only broad policy guidelines regarding the overall role of foods in improving nutrition and food security. This may have contributed to the lack of specific direction and goals in food based strategies.

Even though one can agree that all food based strategies should have an overall long term goal of improving human nutrition, there is an urgent need to quantify goals in this area, giving specific time frames with respect to supplies, access and intakes of specific foods consumed by the poor. This is an important missing tool to enable the formulation and implementation of programmes at different levels with the aim of achieving better defined targets within food based strategies.

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