

important spin-off effects for environmentally beneficial increases in farm productivity for developing countries (Cary et al., 1994; Welch et al., 1993; Kannenberg and Falk, 1995; Graham and Welch, 1996; Graham et al., 1999).

The following section briefly summarizes the extent and consequences of micronutrient malnutrition in developing countries, as well as the effectiveness of the non-agricultural interventions currently being used to address this problem. We then argue that plant breeding is a low-cost, sustainable intervention that can substantially reduce micronutrient malnutrition. This is a new, perhaps not yet widely accepted strategy for addressing this enormous problem, whether using biotechnology or conventional breeding techniques. The progress of such a strategy using conventional breeding techniques is then reported, focusing on rice. Recent advances using biotechnology to improve the micronutrient content of rice are also presented, which permits a discussion of the ways in which conventional and biotechnology approaches are complementary and of what biotechnology can accomplish that conventional breeding cannot. Finally, we draw some general lessons for assessing the potential of biotechnology to improve human nutrition.

2 MICRONUTRIENT MALNUTRITION: EXTENT, COSTS, ALTERNATIVE INTERVENTIONS

Only relatively recently have nutritionists working in developing countries been able to demonstrate that many children and adults, particularly women in their childbearing years, suffer more from a lack of essential vitamins and minerals in their diets than from a lack of calories - even during relative economic and political stability. People are unaware that their diets lack these trace nutrients, and they do not associate these deficiencies with listlessness, poor eyesight, impaired cognitive development and physical growth, and more severe bouts of illness (sometimes leading to death). Accordingly, this general problem of poor dietary quality has been dubbed "hidden hunger". In an observational study, researchers from Johns Hopkins University working in Indonesia showed a correlation between progressively serious eye damage in children and increased child mortality rates. This empirical information was consistent with a long suspected link between vitamin A deficiency and the high child mortality rates common in developing countries. To test this hypothesis more rigorously, 10,000 Indonesian children were given high-dose vitamin A capsules (VAC) and 10,000 children were given a placebo (a low percentage of these children, no more than one percent, had clinically visible eye damage). Mortality rates were found to be 34 percent lower for children who received VAC.

Such a large reduction in mortality was so startling and unexpected that eventually it was necessary to conduct seven similar experiments in Africa and Asia (with similar results on average) before it was widely accepted by the international nutrition community (in the late 1980s) that widespread distribution of VAC could significantly reduce child mortality and should be made a high priority for government intervention. These dramatic, new research findings in the area of vitamin A deficiency also helped to focus more attention and spur further research related to other micronutrient deficiencies, in particular iron and iodine deficiencies.

The World Health Organization (WHO) now compiles statistics on a regular basis about the extent of micronutrient deficiencies and this has revealed the enormous

magnitude of the problem. WHO reported in 1994 that 3 million pre-school age children had eye damage due to a vitamin A deficiency and another 200 million are sub-clinically affected at a severe or moderate level. Annually, an estimated 250,000-500,000 pre-school children go blind from this deficiency, and about two-thirds of these children die within months of going blind. Globally, over three billion people are iron-deficient (ACC/SCN, 2000). The problem for women and children is more severe because of their greater physiological need for iron. In developing countries, more than 40 percent of non-pregnant women and pre-school children and more than one-half of pregnant women have anemia. Of the approximately 500,000 maternal deaths that occur each year due to childbirth, mostly in developing countries, anemia is the major contributor or sole cause in 20-40 percent of such deaths. Iron deficiencies during childhood and adolescence impair physical growth, mental development and learning capacity. In adults, iron deficiency reduces the capacity to do physical labor.

Deficiencies in several other micronutrients, zinc in particular, may be similarly widespread with equally serious consequences for health (Gibson, 1994). However, because there are no specific indicators to screen for zinc deficiencies (other than a positive health response to supplementation), zinc has not received as much attention. The costs involved in fortification and supplementation are considerable. The recurrent, annual, lower-bound estimate for iron supplementation is US \$2.65 per person when administrative costs are taken into account (Levin et al., 1993). A lower-bound estimate for iron fortification is 10 cents per person per year. In a populous country such as India (total population 1 billion) there may be as many as 28 million anemic pregnant women in any given year. Treating only half of those women through a well-targeted supplementation program could cost as much as US \$37 million per year. Iron fortification for half the entire population could cost \$44 million per year. Notwithstanding these cost estimates, the benefits of properly managed interventions can be quite significant.

The World Bank's World Development Report 1993 found that micronutrient programs were among the most cost-effective of all health interventions. A World Bank document (1994) estimates that deficiencies of just vitamin A, iodine, and iron alone could waste as much as 5 percent of gross domestic product (GDP) in developing countries, but addressing them comprehensively and sustainably would cost less than one-third of a percent of GDP. Nevertheless, it is difficult for governments and international agencies to mobilize resources of this magnitude.

Although successful supplementation and fortification do not require a substantial change in individual behavior, these interventions only treat the symptoms and not the underlying causes of micronutrient deficiencies. This has led many to advocate the use of "food-based" interventions, such as nutrition education and the promotion of home vegetable gardens, that address the underlying cause of poor quality diets and also provide a range of other important nutrients. This approach, however, means changing human behavior, which can be both expensive and difficult. New and compelling scientific evidence is rapidly accumulating to support the claim that nutrition and health in developing countries can be dramatically improved by reducing micronutrient malnutrition. It is an enormous opportunity. Nevertheless, there is some frustration at the lack of appropriate, well-developed tools for

developing countries to solve the problem of micronutrient deficiencies quickly and at a reasonable cost.

3 CAN A PLANT BREEDING STRATEGY WORK? FIVE KEY QUESTIONS

A strategy of breeding plants that enrich themselves and load high amounts of minerals and vitamins into their edible parts has the potential to substantially reduce the recurrent costs associated with fortification and supplementation. But this will be successful only if farmers are willing to adopt such varieties, if the edible parts of these varieties are palatable and acceptable to consumers, and if the incorporated micronutrients can be absorbed by the human body. Indeed, five core questions must be addressed to examine the feasibility of such a plant breeding strategy.

3.1 Is it Scientifically Feasible to Breed Micronutrient-Dense Staple Food Varieties?

At least three agricultural research projects in developed countries have successfully manipulated the mineral uptake of plants and the mineral content of plant seeds, and all these projects have been commercially successful. Zinc-dense wheat varieties, developed at the Waite Agricultural Research Institute of the University of Adelaide, are already being grown on a commercial basis in Australia (Rengel and Graham, 1995). In the United States, an iron-efficient soybean has been developed to overcome problems of iron "deficient" soils, and cadmium levels in durum wheats have been reduced through plant breeding to meet quality standards in countries importing US wheat.

3.2 What are the Effects on Plant Yields? Will Farmers Adopt Such Varieties?

Results from research at Waite and elsewhere has shown that where the soil is deficient in a particular micronutrient, seeds containing more of that nutrient have better germination, better seedling vigor and/or more resistance to infection during the vulnerable seedling stage (Pearson and Rengel, 1995; McCay et al., 1995). Since these crop establishment benefits can result in higher crop yield, the specific breeding goals for human and plant nutrition largely coincide. A soil is said to be "deficient" in a nutrient when the addition of a fertilizer containing that nutrient produces better growth. But the amount of the mineral micronutrient that is added to the soil to improve growth is usually small compared to the total amount of that mineral found in the soil. Because the trace mineral is chemically bound to other elements in the soil, the major part of the trace mineral is "unavailable" to plants. An alternative view, therefore, is that instead of soil deficiency there is a genetic deficiency in the plant. Tolerance to micronutrient-deficient soils, termed micronutrient efficiency, is a genetic trait of a genotype or phenotype that causes a plant to be better adapted or to produce higher yields in a micronutrient-deficient soil than the average cultivar of the species (Graham and Rovira, 1984). Growing zinc-efficient plants on zinc-deficient soils, for example, "tailors the plant to fit the soil" instead of "tailoring the soil to fit the plant" (Foy, 1983). These efficient genotypes exude substances from their roots that chemically unbind trace minerals from other binding elements and make trace minerals available to the plant.

It is well understood that without replacement the depletion of soil nitrogen takes only a few years. Consequently, it is pointless to breed for greater tolerance to nitrogen-deficient soils. Phosphorus efficiency results in overall improvements in cost-efficiency but, without replenishment, depletion of soil phosphorous will also eventually occur. In contrast, the depletion of mineral micronutrients may take hundreds or thousands of years - or may likely never occur at all - due to various inadvertent additions and other processes, such as minerals carried in windblown dust (Graham, 1991). Based on a number of soil surveys, particularly in China where the most extensive surveys have been done, it is estimated that at least 50 percent of the arable land used for crop production worldwide is low in one or more of the essential micronutrients. For example, although iron is the fifth most abundant element in the earth's crust, the fraction of soil iron that is in soluble form for absorption by plants may only be 10 -13 of total soil iron.

Depletion of soil iron is never an issue; instead, the issue is the ability of the plant to mobilize sufficient iron to satisfy its needs (Han et al., 1994). Zinc deficiency is probably the most widespread micronutrient deficiency in cereals. Sillanpaa (1990) found that 49 percent of a global sample of 190 soils in 25 countries were low in zinc. Unlike other micronutrients, zinc deficiency is a common feature of both cold and warm climates, in soils drained and flooded, acid and alkaline, heavy and light (Rahman et al., 1993).

Good nutrition balance is as important for disease resistance in plants as it is in humans. The efficient uptake of mineral micronutrients from the soil is associated with disease resistance in plants, which leads to decreased use of fungicides. Micronutrient deficiency in plants greatly increases their susceptibility to diseases, especially fungal root diseases of the major food crops. The picture emerging from four decades of physiological studies of roots is that phosphorus, zinc, boron, calcium and manganese are all required in the external environment of the root for membrane function and cell integrity. In particular, phosphorus and zinc deficiencies in the external environment promote the leaking of cell contents such as sugars, amides and amino acids. These substances are chemotoxic stimuli to pathogenic organisms. It also appears that micronutrient deficiency predisposes the plant to infection, rather than the infection causing the deficiency through its effect on root pruning (Sparrow and Graham, 1988; Thongbai et al., 1993). Breeding for micronutrient efficiency can also confer resistance to root diseases that had previously not been amenable to breeding solutions. This could mean a lower dependence on fungicides. Micronutrient-efficient varieties grow deeper roots in mineral deficient soils and are better at tapping subsoil water and minerals (Grubb, 1994; Brown et al., 1994). When topsoil dries, roots in the dry soil zone (which are not tightly linked to agronomic zinc efficiency traits and may have to be selected for independently).

3.3 Will Micronutrient-Density: Change the Consumer Characteristics?

Mineral micronutrients comprise a tiny fraction of the physical mass of a seed, perhaps ten parts per million. Dense seeds may contain perhaps as many as fifty parts per million. It is not expected that such small amounts will alter the appearance, taste, texture or cooking quality of foods. Increasing the seed content of beta-carotene, which is associated with an orange or yellow color, will alter its color. This might well reduce consumer preference, but nutrition education could turn this obstacle to

an advantage, as consumers could be taught that deepness of color indicates a nutrient-dense product.

3.4 Will the Extra Micronutrients in Staple Foods be Bioavailable?

An underlying cause and fundamental constraint to solving the micronutrient malnutrition problem is that non-staple foods, particularly animal products, tend to be the foods richest in bioavailable micronutrients. These are precisely the foods that the poor in developing countries cannot afford. Their diets consist mostly of staple foods, primarily cereals. For the poor, these staple foods already are primary sources of what micronutrients they are able to consume, particularly minerals. This is demonstrated by food intake data shown in Table 1 for survey populations in Bangladesh and the Philippines. Average incomes in these households range from US \$45 per capita per year in the poorest 20 percent of households to \$250 in the richest 20 percent of households. Thus, they are typical of the middle to lower end of the income distribution in the rural areas of these countries. The first priority for these poor households in terms of food purchases is to obtain calories to satiate hunger. The most inexpensive sources of calories in Bangladesh are rice and wheat; in the Philippines they are maize and rice. Once a critical amount of calories are acquired from inexpensive food staples, if income is available, consumers purchase non-staple foods at the margin, particularly animal products and fruits, and to some extent substitute more expensive, more preferred food staples for inexpensive staples. Not only are food staples poor (non-dense) sources of trace minerals, but anti-nutrient (e.g., phytic acid) levels are high, which reduces the overall human health. Therefore, they argue against a breeding strategy that attempts to increase iron bioavailability by reducing anti-nutrient content. Raboy (1996), however, has developed low phytic acid (or lpa) mutant varieties of maize, rice and barley. The phytic acid content of lpa seeds is reduced by 50-80 percent as compared with non-mutant seeds. The total amount of phosphorus remains the same - phytic acid is replaced by inorganic phosphorus, which does not bind a range of trace minerals.

These mutations typically have little observable effect on other seed or plant characteristics. These varieties are presently being tested for agronomic performance and effects on micronutrient status in humans. Promoters of bioavailability. Certain amino acids (cysteine and lysine, but particularly methionine) enhance iron and/or zinc bioavailability (Hallberg, 1981). These amino acids occur in many staple foods, but their concentrations are lower than those found in meat products. A modest increase in the concentrations of these amino acids in plant foods may have a positive effect on iron and zinc bioavailability in humans. Iron and zinc occur only in micromolar amounts in plant foods, so only micromolar increases in the amounts of these amino acids may be required to compensate for the negative effects of anti-nutrients on iron and zinc bioavailability. These amino acids are essential nutrients for plants as well as for humans, so relatively small increases of their concentrations in plant tissues should not have adverse consequences on plant growth .

Table 1: (unable to reproduce the table!!)

Nevertheless, Table 1 reveals that primary food staples provide about 40-55 percent of total iron intakes for lower income households. If a single food staple provides 50 percent of total iron intakes for a poor population (e.g., rice in Bangladesh), then a

doubling of the iron density in that food staple will increase total iron intakes by 50 percent, and tripling the iron density will double total iron intakes. One strength of a plant breeding approach that focuses on food staples, therefore, is that it relies on existing consumer behavior. The poor consume large amounts of food staples on a daily basis. If a high proportion of the domestic production of food staples can be provided by nutritionally improved varieties, nutritional status can be improved without resorting to programs that depend on behavioral change.

A key issue is whether the bioavailability percentage of total iron (or zinc) intakes will remain constant or decline. Rat studies suggest that the percent of bioavailable iron (and zinc) remains relatively constant across cereal genotypes with high and low density (Welch, 1996; Welch et al., 1996). Likewise, if nutritionally-improved varieties have unique agronomic advantages in trace mineral-deficient soils, or if these traits are incorporated into highly profitable varieties, then no behavioral change is required of farmers since profits will motivate them to adopt and produce these nutritionally-improved varieties.

3.5 Are there Cheaper or Easier Sustainable Strategies for Reducing Micronutrient Malnutrition?

A plant breeding strategy, if successful, will not eliminate the need for supplementation, fortification and dietary diversification programs in the future. Nevertheless, by significantly reducing the numbers of people requiring treatment, this strategy has the promise to significantly reduce the recurrent expenditures required for these higher-cost, short-run programs. Costs of plant breeding. To obtain a rough estimate of plant breeding costs, the example of the CGIAR Micronutrients Project may be used. This project is a multi-disciplinary effort among plant scientists, human nutritionists and social scientists. The general objective over five years is to assemble the package of tools that plant breeders will need to produce mineral- and vitamin-dense cultivars. The target crops are wheat, rice, maize, phaseolus beans and cassava. The target micronutrients being studied are iron, zinc and vitamin A. When such mutants are used as animal feeds, this also avoids what has become a serious pollution problem: excretion of unutilized phytic acid. The plant breeding effort can be seen as a two-stage process. The first five-year phase primarily involves research at central agriculture research stations, at an estimated US \$2 million per year for research on all five crops. During this initial phase, promising germplasm is identified and the general breeding techniques are developed for later adaptive breeding. During the second phase, the emphasis shifts to national agricultural research. Total costs and duration of this second phase are difficult to estimate, but will depend on the number of countries involved and the number of crops worked on in each country. The annual cost for each country should not be more than the US \$2 million per year estimated for the first phase.

Benefits to improved human nutrition: The World Bank (1994) estimates that at the levels of micronutrient malnutrition existing in South Asia, 5 percent of gross national product is lost each year due to deficiencies in the intakes of just three nutrients: iron, vitamin A and iodine. For a hypothetical country of 50 million persons burdened with this rate of malnutrition, deficiencies in these three nutrients could be eliminated through fortification programs costing a total of US \$25 million annually, or 50 cents per person per year. The monetary benefit to this \$25 million investment is quite high

in terms of increased productivity - estimated at \$20 per person per year, or a forty-fold return on an investment of 50 cents. These benchmark numbers will be used below as a basis of comparison with the benefits of a plant breeding strategy.

Calculation of benefit-cost ratios: The details of a formal benefit-cost analysis are presented in Bouis (1999). Expressed in present values, costs are about US \$13 million and benefits \$274 million, giving a benefit-cost ratio of over 20, which is quite favorable despite the very conservative assumptions made and despite the long time lag between investments and benefits. This last point highlights an essential difference between investments in standard fortification programs and fortification through plant breeding strategies. Standard fortification programs must be sustained at the same level of funding year after year. If investments are not sustained, benefits disappear. Such investments apply to a single geographical area, such as a nation-state. By contrast, research investments in plant breeding have multiplicative benefits that may accrue to a number of countries. Moreover, these benefits are sustainable, since as long as an effective domestic agricultural research infrastructure is maintained, breeding advances typically do not disappear after initial investments.

4 FINDINGS-TO-DATE OF THE CGIAR MICRONUTRIENTS PROJECT

Convinced that the existing scientific evidence provided satisfactory answers to the above five questions, since 1995 the Danish Development Agency (DANIDA), the US Agency for International Development (USAID), and the Australian Council for International Agricultural Research (ACIAR) have funded exploration of the potential for micronutrient density in CGIAR germplasm banks of the major staple crops. A report was published recently for several food staples (Graham et al., 1999). A summary of these findings with respect to rice is presented below. Rice. The findings in the rice component of the project are particularly encouraging. Iron density in unmilled rice varied from 7-24 parts per million (ppm or mg/kg) and zinc density from 16-58 ppm. Because nearly all the widely grown "green revolution" varieties were similar, a benchmark was established of about 12 and 22 ppm for iron and zinc, respectively. The best lines discovered in the survey of the germplasm collection were therefore twice as high in iron and 1.5 times as high in zinc as the most widely grown varieties today. High iron and to a lesser extent, high zinc concentration, were subsequently shown to be linked to the trait of aromaticity. Most aromatic rices, such as jasmine and basmati, are high in iron, zinc and generally in most minerals (Senadhira and Graham, 1999; Graham et al., 1997; Graham et al., 1999). The close linkage to aroma suggests iron density in rice expresses as a single gene trait since aroma is itself controlled at a single locus.

As in other crops, these micronutrient density traits have been combined with high yield. A promising aromatic variety found to be high in iron, designated IR68-144, is already being tested at IRRI due to its superior agronomic and consumer characteristics. This aromatic variety has double the iron (after milling) of standard IRRI releases and is early maturing, high yielding and disease resistant. Bioavailability tests using human subjects are planned to begin in 2000. Pending the results of these bioavailability tests and agronomic tests to be undertaken by the Philippine Seed Board, IR68-144 may be ready for release to farmers in the Philippines in a few years. Genotype environment interactions. Expression of the micronutrient-density traits has been tested over a wide range of environments, and

although the environmental effect itself is strong, the genotype effect is consistent across environments and sufficient to encourage a breeding effort. Environmental factors considered by one or more of the crop programs include acid soils, alkaline soils, saline soils, acid-sulfate soils, iron-deficient soils, time of planting, field site, season, nitrogen fertilization, phosphorus fertilization, potassium status, elevation and drought stress.

5 RECENT FINDINGS ON IMPROVING THE NUTRIENT CONTENT OF RICE USING BIOTECHNOLOGY

5.1 Increasing Iron Content:

Ferritin is an iron-storage protein found in animals, plants and bacteria. The ferritin gene has been isolated and sequenced in plants, including soybean, French bean, pea and maize. Recent studies show that both plants and animals use ferritin as the storage form of iron and that, orally administered, it can provide a source of iron for treatment of rat anemia (Beard et al., 1996). However, human studies with extrinsically radiolabeled animal ferritin have indicated that iron contained within the ferritin molecule added to a meal is only about half as well absorbed as vegetable iron (Martinez-Torres et al., 1976; Taylor et al. 1986) and as ferrous sulphate (Skikne et al., 1997).

As yet, there have been no human studies with plant ferritin, and animal studies are not considered a good model for humans (Hurrell, 1997). Goto et al. (1999) report improving the iron content of rice by transferring the entire coding sequence of the soybean ferritin gene into a Japonica rice. The introduced ferritin gene was expressed under the control of a rice seed-storage protein glutelin promoter to mediate the accumulation of iron specifically in the grain. The transgenic seeds stored up to three times more iron than the normal seeds. Iron levels in the unmilled seeds of the transformants varied from 13 to 38 ppm, while that of the non-transformants varied from only 9 to 14 ppm. Pooled mean values were 23 ppm for transformants and 11 ppm for non-transformants. The average iron content in the endosperm of the transformant was 3.4 ppm and 1.6 ppm in the non- 3 The authors state that the iron content in a meal-size portion of ferritin rice (approximately 5.7 mg-Fe/150 g dryweight) would be sufficient to provide 30-50 percent of the daily adult iron requirement. 5.7 mg-Fe is presumably obtained by multiplying 38 ppm by 150 g. Unfortunately, this calculation assumes that consumers eat unmilled rice. The iron added by the ferritin rice in the endosperm is 1.8 ppm (3.4 ppm minus 1.6 ppm), which when multiplied by 150 g, gives only an extra 0.27 mg Fe per meal-sized portion. Nevertheless, this alternative calculation probably understates the iron added because milling does away with much, but not all of the seed's brown outer covering (bran) that is relatively dense in iron.

In a heavy rice-eating population, an adult may consume 400 g (1,400 kcal) of rice (dryweight) a day. If the differential in iron content in milled rice is 10 ppm between an iron-dense (say 18 ppm) and normal-iron rice (say 8 ppm), this confers an additional 4 mg of iron to the diet per day, which may be a 50 percent increase over the average daily intake of a poor person who obtains 80-90 percent of their energy from rice. This underscores the importance of determining where in endosperm the iron (and other trace minerals) are deposited and how mineral levels are affected by

milling. (??) transformant. The authors speculate that the amount of iron accumulation is restricted by the transport of iron to the ferritin molecule, rather than simply by levels of ferritin protein. It may be possible, therefore, to store larger amounts of iron in the ferritin molecule by cointegrating the ferritin gene and the iron reductase-like transporter gene.

Although results have yet to be published in a refereed journal, at a recent conference Ingo Potrykus and colleagues at the Swiss Federal Institute of Technology announced a doubling of the iron content in a rice using a ferritin gene derived from *Phaseolus vulgaris* (cf. Gura, 1999). Metallothioneine was also expressed in the rice grain, increasing the cysteine content seven fold. It is not known if the cysteine containing peptides formed on digestion of metallothioneine in the human gut have a similar enhancing effect on iron absorption as those formed on digestion of muscle tissue (Hurrell, personal communication).

5.2 Introducing a Heat-Stable Phytase Gene Which Breaks Down Phytic Acid:

The phytase level in rice is normally low. Several studies have already demonstrated the usefulness of adding phytase to the rice diets of poultry (e.g., Adrizal et al., 1996; Farrell and Martin, 1998; Martin et al., 1998). The phytase found in rice seeds will hydrolyze phytic acid if seeds are soaked in water. However, boiling destroys the phytases that occur naturally in rice. The research team led by Potrykus also reported introducing a transgene for a heat-stable phytase from *Aspergillus fumigatus*, which increased the level of phytase 130-fold. The fact that the phytase is potentially heat-stable, then, is critically important. An amino acid had been changed in the sequence to make the phytase heat stable (Pasamontes, 1997). It was also active under the conditions (pH) of digestion and degraded all the phytic acid in a very short time during model in vitro digestion. Unfortunately, after expression in the grain it was no longer stable to heat and lost its activity on boiling (Hurrell, personal communication).

5.3 Increasing Promoters Levels of lysine, an essential but limiting amino acid in rice that might promote the uptake of trace minerals, can be increased by genetic engineering (Datta, 1999). The introduction of two bacterial genes DHSPS (dihydrodipicolinic acid synthase) and AK (aspartokinase) enzymes encoded by the *Corynebacterium* *dapA* gene and a mutant *E. coli* *lysC* gene have enhanced lysine about five-fold in canola and soybean seeds (Falco et al., 1995).

5.4 Adding Beta-Carotene:

Beta-carotene, a precursor of vitamin A (retinol), does not occur naturally in the endosperm of rice. Ye et al. (2000) have reported generating a large series of transgenic plants that produce grain with yellow-colored endosperm. Biochemical analysis confirmed that the color represents beta-carotene (provitamin A). According to Ye et al. (2000), *psy* (cloned from *Narcissus pseudonarcissus*; Schledz et al., 1996), *crt1* (cloned from *Erwinia uredovora*; Misawa et al., 1993), and the *lyc* gene have been introduced into the rice, driven by the endosperm specific glutelin promoter (*Gt1*). *Crt1* was fused to the transit peptide (*tp*) sequence by the pea Rubisco small sub-unit (Misawa et al., 1993) to lead the accumulation of lycopene in the endosperm plastids. This is a remarkable accomplishment considering that most traits engineered to date have only required the addition of a single gene (Guerinot, 2000). The reported level of beta-carotene in one gram of the transformed rice is 1.6 mg. Multiplying that

by a daily intake of 400 grams of milled rice and dividing by a conversion ratio of 6 mg of beta-carotene for every retinol equivalent (RE) gives 107 RE, which Ye et al. (2000) state is the target level for improved nutrition. However, widely accepted RDAs for vitamin A range between 375-1,200 RE depending on age, gender and physiological status, and recent evidence suggests that the conversion factor from beta-carotene to RE varies between 12-26 to 1 (de Pee et al., 1998). Nevertheless, RDAs are set relatively high by adding two standard deviations to the observed mean requirements of a nutrient for most people.

According to Datta (1999), the introduction of ferritin, heat-stable phytase and beta-carotene in rice by the Potrykus-led team has been accomplished individually in separate Japonica rices (i.e., not jointly in the same rice). These cultivars may be used in the IRRI breeding program to transfer the genes of interest to Indica cultivars from which IRRI releases are derived. Alternatively, these genes can be introduced directly into Indica cultivars using biotechnology. Ye et al. (2000) use a ration of 300 g of milled rice in their calculations but add that they are optimistic that they can reach a goal of 2 mg/g of beta-carotene in homozygous lines. In a relatively heavy-rice-eating population such as Bangladesh, non-breastfeeding pre-schoolers in rural areas might consume 250 g of milled rice per day (an RDA of 500 RE), adult women 500 g of milled rice per day (an RDA of 800 RE if not pregnant or breastfeeding), and adult men 650 g of milled rice per day (an RDA of 1,000 RE).

6 STRATEGY FOR COMBINING CONVENTIONAL BREEDING AND BIOTECHNOLOGY

What is the appropriate mix of conventional breeding techniques and biotechnology in breeding for micronutrient-dense staple food crops? Where are they complementary? Where is one approach feasible, but the other not? Table 2 summarizes some of the issues involved. To return to two of the themes raised earlier in the paper, the fundamental advantages of breeding for increased trace minerals are that (i) agricultural productivity is not compromised, indeed is enhanced on trace mineral deficient soils, and (ii) consumer characteristics should remain unchanged. Thus, for rice, although it is fortunate that IR68-144 was "discovered" without resort to breeding explicitly for high iron, such a discovery has a relatively high probability of occurring because of the compatibility with high yields of the nutritional trait being sought. Such "discoveries" can greatly speed up the development and dissemination process and lower the cost of breeding. After milling, IR68-144 confers perhaps an 80 percent increase in iron density over modern varieties presently being released. This is about the same average advantage as reported by Goto et al. (1999) and the Potrykus group. It may be possible to further elevate this 80 percent advantage by crossing various iron-dense genotypes that may have complementary, additive mechanisms for loading more iron into the seed. The two successes at adding a ferritin gene to rice reported here have not given superior results to those obtained by conventional plant breeding. Moreover, breeding is still required to move the "ferritin" trait from Japonica to high-yielding Indica rice varieties. It may be that eventually the most iron-dense genotypes can be developed using biotechnology, especially after the basic physiology of what mechanisms control translocation of trace minerals in plants is better understood. However, a specific strategy for doing so has yet to be demonstrated.

Goto et al. (1999) and Potrykus measure their iron increases against benchmarks that are the non-transformed genotypes. But are these benchmarks relatively high or low density genotypes to start with? The only figures available are from Goto et al. (1999). Their maximum value of 38 ppm in brown rice is quite high compared with all rices analyzed under the CGIAR Micronutrients Project. However, this was an analysis of only one single grain from a plant grown under laboratory conditions (iron content varies by weather and soil type). Analyses under the CGIAR Micronutrient Project are averages for randomly drawn samples of several seeds of a single genotype grown in a specific season on a specific soil. Thus, the average of 23 ppm obtained by Goto et al. (only 11 grains in total) is probably the best comparison with IR68-144, which has an iron density of 23 ppm for some plantings (Gregorio, 1999).

===== Table 2: (not reproduced) =====

We conclude that, in the short-to-medium term, conventional breeding methods may give superior results with respect to iron and zinc density as compared to biotechnology. However, the implication is not that biotechnology-related, long-term research to increase trace mineral density should be stopped. Rather, spending using either methodology is presently quite low and should be increased in view of the cost-benefit analysis presented earlier.

Three complementary or alternative approaches to increasing the bioavailability of trace minerals in the grains of food staples are listed in Table 2: (i) reducing phytic acid, (ii) introducing phytase, and (iii) increasing promoting compounds.

As described earlier, low phytic acid mutants of rice, maize, wheat and barley have already been produced in which virtually all or a large portion of the phytic acid has been replaced by inorganic phosphorus. This is a promising approach in terms of improving human nutrition, although a drawback may be its agronomic performance on phosphorus-poor soils. Evidence about which compounds promote bioavailability, such as sulfur-containing amino acids, is sketchy. Until this approach is more thoroughly researched and specific compounds firmly identified as promoting bioavailability, it is probably too early to begin breeding for such compounds using conventional plant breeding or biotechnology. A promising approach for the use of biotechnology with respect to trace minerals is to add a heat-stable phytase to rice. This particular approach is not an option for conventional plant breeding and can only be pursued using biotechnology. No data exist on how adding phytase to rice would affect agronomic performance or consumer characteristics. Turning now to vitamins, the addition of beta-carotene in rice is possible only through biotechnology. No rice has been identified with beta-carotene in the endosperm.

Thus, the apparent success of Potrykus' group in this regard is quite exciting. Nevertheless, apart from breeding these beta-carotene-related genes into high-yielding varieties and assessing possible effects on plant productivity, it is already known that the beta-carotene turns the seeds yellow. How willingly will poor consumers purchase and consume yellow rice? Most people agree that without a complementary nutrition education program, consumers will not readily accept "yellow rice." Opinions differ as to the power of nutrition education to overcome culturally held preferences for white rice. It is easy to speculate either way, but no hard data are available. If a nutrition education program were successful, however, the

yellow color would distinguish more nutritious rice from less nutritious rice and the disadvantage of the yellow color would be turned to an advantage.

An additional exciting possibility is that higher intakes of beta-carotene (converted to retinol after ingestion) may promote absorption of iron and vice versa. That is, there are possible synergies between higher intakes of these two nutrients (Garcia-Casal et al., 1998). There is already considerable evidence about the synergies between vitamin A and zinc intakes (Smith, 1996).⁶ According to Preben Holm (personal communication), plant phytases have been very difficult to isolate. For cereals there are only two maize phytase genes isolated, one expressed in the seedling and the other in the root. This implies that there are virtually no tools available for gene expression analyses and for immuno detection of proteins.

7 LESSONS TO BE DRAWN

The following lessons may be drawn concerning the potential usefulness of biotechnology in helping to provide more nutritious food staples in developing countries:

1. It must be established that plant breeding is more cost-effective than alternative interventions already in place to reduce micronutrient malnutrition. This is apparently the case, in large measure because of the multiplier effects of plant breeding - a relatively small, fixed, initial investment in research may benefit the health of millions of poor people in developing countries all over the world, at the same time improving agricultural productivity on lands which are presently among the least productive.
2. There must be aspects of the breeding strategy for which biotechnology is superior to conventional breeding techniques. For rice, this is the case for adding beta-carotene-related and heat-stable phytase genes. In the long run, as more is understood about the factors driving translocation of minerals in plants, it may also be helpful for increasing trace mineral density. However, present evidence suggests that in the short-run, conventional breeding techniques work as well and may be applied more quickly.
3. In those areas of plant breeding where biotechnology is superior to conventional plant breeding, it must be established that: (i) there are no serious, negative agronomic consequences associated with the characteristic being added; (ii) consumers will accept any noticeable changes in the color, taste, texture, cooking qualities and other features associated with the characteristic being added; and (iii) the characteristic being added will measurably improve the nutritional status of the malnourished target population. The conditions under lesson three, in particular, have yet to be firmly established, but it is important not to be overly cautious. The potentially enormous benefits to the poor in developing countries in relation to costs are so high that research in this area should be vigorously pursued.

8 CONCLUSION

Biotechnology can improve consumer welfare in a number of ways not discussed in this paper. By helping to improve crop and animal productivity and thereby increasing the growth rate of supply of a range of foods, biotechnology can help reduce food prices for poor consumers. Lower cereal prices can have substantial income effects

for such consumers, improving their ability to purchase non-staple foods that are rich in bioavailable minerals and vitamins. Lower prices for non-staple foods themselves, of course, also permit higher consumption of micronutrient-dense foods. Consumer-preferred characteristics of food, such as appearance, taste and color, can also be improved through biotechnology, but such research probably benefits rich consumers more than poor consumers in developing countries.

Ultimately, good nutrition depends on adequate intakes of a range of nutrients and other compounds in combinations and levels that are not yet completely understood. The best and final solution to malnutrition in developing countries is to provide increased consumption of a range of non-staple foods. By reducing the cost of producing food, biotechnology will perhaps make its most important contribution to reducing malnutrition. However, this will require several decades, informed government policies and a relatively large investment in agricultural research and other public and on-farm infrastructure to be realized.

In the medium run, a much smaller investment in breeding nutrient-dense staple foods can make a major contribution to reducing deficiencies in selected micronutrients. Because of the inherent compatibility of high yields and trace mineral density, some successes in increasing the mineral content of staples can be achieved in the short-run through conventional breeding techniques. Plant breeding is a new strategy for improving nutrition, and it is essential to make these early, nutritionally improved varieties available to farmers for commercial production. Any resulting improvements in micronutrient status must be measured to demonstrate the feasibility and practicality of plant breeding for improving micronutrient nutrition. Once feasibility and practicality are established for specific crops and nutrients, the hope is that donor agencies will decide to accept the relatively long lead times involved in plant breeding strategies and that these agencies and agricultural research systems will adequately fund the required research. The full potential of biotechnology can then be applied to improving the nutritional content of food staples by (i) perhaps increasing mineral levels even further than is possible with conventional breeding techniques and (ii) pursuing complementary strategies, such as adding beta-carotene and heat-stable phytase genes, that are not possible using conventional breeding techniques.

REFERENCES

ACC/SCN (Administrative Committee on Coordination, Subcommittee on Nutrition) and International Food Policy Research Institute (2000): Fourth Report on the World Nutrition Situation. United Nations, Geneva. Adrizal, P., E. Palo, and J.L. Sell (1996): Utilization of Defatted Rice Bran by Broiler Chickens. *Poultry Science* 75 (8), pp. 1012-1017.

Beard, J.L., J.W. Burton, and E.C. Theil (1996): Purified Ferritin and Soybean Meal Can Be Sources of Iron For Treating Iron Deficiency in Rats. *Journal of Nutrition* 126, pp. 154-160.

Bouis, H.E. (1999): Economics of Enhanced Micronutrient Density in Food Staples. *Field Crops Research* 60, pp. 165-173.

Bouis, H.E., and L.J. Haddad (1990): Agricultural Commercialization, Nutrition, and

the Rural Poor: A Study of Philippine Farm Households. Lynne Rienner Publishers, Boulder. Bouis, H.E., and M.J. Novenario-Reese (1997): The Determinants of Demand for Micronutrients: An Analysis of Rural Households in Bangladesh. FCND Discussion Paper, International Food Policy Research Institute, Washington, D.C. Brown, P.H., Q. Zhang, and L. Ferguson (1994): Influence of Rootstock on Nutrient Acquisition by Pistachio. *Journal of Plant Nutrition* 17, pp. 1137-1148.

Cary, E.E., W.A. Norvell, D.L. Grunes, R.M. Welch, and W.S. Reid (1994): Iron and Manganese Accumulation by the brz Pea Mutant Grown in Soils. *Agronomy Journal* 86, pp. 938-941.

Datta, S.K. (1999): The Genetic Engineering Approach to Development of Nutritious Rice. Paper presented at the workshop "Improving Human Nutrition Through Agriculture: The Role of International Agricultural Research", 5-7 October, Los Baños, Philippines. Falco, S.C., T. Guida, M. Locke, J. Mauvais, C. Sanders, R.T. Ward, and P. Webber (1995): Transgenic Canola and Soybean Seeds with Increased Lysine. *Biotechnology* 13, pp. 577-582.

Farrell, D.J., and E.A. Martin (1998): Strategies to Improve the Nutritive Value of Rice Bran in Poultry Diets, III; The Addition of Inorganic Phosphorus and a Phytase to Duck Diets. *British Poultry Science* 39 (5), pp. 601-611.

Foy, C.D. (1983): Plant Adaptation to Mineral Stress in Problem Soils. *Iowa State Journal of Research* 57, pp. 355-391. Garcia-Casal, M.N., M. Layrisse, L. Solano, F. Arguello, D. Llovera, J. Ramírez, I. Leets, and E. Tropper (1998): Vitamin A and b-Carotene Can Improve Nonheme Iron Absorption from Rice, Wheat and Corn by Humans. *Journal of Nutrition* 128, pp. 646-650.

Gibson, R.S. (1994): Zinc Nutrition in Developing Countries. *Nutrition Research Reviews* 7, pp. 151-173.

23 Goto, F., T. Yoshihara, N. Shigemoto, S. Toki, and F. Takaiwa (1999): Iron Fortification of Rice Seed By the Soybean Ferritin Gene. *Nature Biotechnology* 17, pp. 282-286.

Graham, R.D., and A.D. Rovira (1984): A Role for Manganese in the Resistance of What to Take-All. *Plant Soil* 78, pp. 441-444.

Graham, R.D. (1991): Breeding Wheats for Tolerance to Micronutrient Deficient Soil: Present Status and Priorities. In: D.A. Saunders (ed.). *Wheat for the Nontraditional Warm Areas*. International Maize and Wheat Improvement Center, Mexico City. Graham, R.D., D. Senadhira, and I. Ortiz-Monasterio (1997): A Strategy for Breeding Staple-Food Crops with High Micronutrient Density. *Soil Science Plant Nutrition* 43, pp. 1153-1157.

Graham, R.D., D. Senadhira, S.E. Beebe, C. Iglesias, and I. Ortiz-Monasterio (1999): Breeding for Micronutrient Density in Edible Portions of Staple Food Crops: Conventional Approaches. *Field Crop Research* 60, pp. 57-80.

Graham R.D., and R.M. Welch (1996): Breeding for Staple Food Crops With High Micronutrient Density. *Agricultural Strategies for Micronutrients*, Working Paper 3, International Food Policy Research Institute, Washington, D.C. Gregorio, G. (1999): Research on Trace Minerals in Rice. Paper presented at the workshop "Improving Human Nutrition Through Agriculture: The Role of International Agricultural Research," 5-7 October, Los Baños, Philippines. Grubb, P.J. (1994): Root Competition in Soils of Different Fertility: A Paradox Resolved? *Phytocoenologia* 24, pp. 495-505.

Guerinot, M.L. (2000): The Green Revolution Strikes Gold. *Science* 287, pp.241-243.

Gura, T. (1999): New Genes Boost Rice Nutrients. *Science* 285, pp. 994-995. Hallberg, L. (1981): Bioavailability of Dietary Iron in Man. *Annual Review of Nutrition* 1, pp. 123-127.

Han, Z.H., Q. Wang, and T. Shen (1994): Comparison of Some Physiological and Biochemical Characteristics Between Iron-Efficient and Iron-Inefficient Species in the Genus *Malus*. *Journal of Plant Nutrition* 17, pp. 1257-1264.

Hurrell, R.F. (1997): Bioavailability of Iron. *European Journal of Clinical Nutrition* 51 (Supplement 1), pp. 4-8.

Kannenbergh, L.W., and D.E. Falk (1995): Models for Activation of Plant Genetic Resources for Crop Breeding Programs. *Canadian Journal of Plant Sciences* 75, pp. 45-53.

Levin, H., E. Pollitt, R. Galloway, and J. McGuire (1993): Micronutrient Deficiency Disorders. D. Jamison, W. Mosley, A. Measham, and J. Bobadila (eds.). *Disease Control Priorities in Developing Countries*. Oxford University Press, New York. Martin, E.A., J.V. Nolan, Z. Nitsan, and D.J. Farrell (1998): Strategies to Improve the Nutritive Value of Rice Bran in Poultry Diets, IV; Effects of Addition of Fish 24 Howarth E. Bouis Meal and a Microbial Phytase to Duckling Diets on Bird Performance and Amino Acid Digestibility. *British Poultry Science* 39 (5), pp. 612-621.

Martinez-Torres, C., M. Renzi, and M. Layrisse (1976): Iron Absorption By Humans from Hemosiderin and Ferritin, Further Studies. *Journal of Nutrition* 106, pp. 128-135. McCay, B.T.S., D.M. Huber, R.D. Graham, J.D. Phillips, and K.E. Miskin (1995): Manganese Seed Content and Take-All of Cereals. *Journal of Plant Nutrition* 18, pp. 1711-1721.

Misawa, N., S. Yamono, H. Linden, M.R. de Felipe, M. Lucas, H. Ikenga, and G. Sandmann (1993): Functional Expression of the *Erwinia Uredovora* Carotenoid Biosynthesis Gene *crtl* in Transgenic Plants Showing an Increase of *b*-Carotene Biosynthesis Activity and Resistance to the Bleaching Herbicide Norflurazon. *Plant Journal* 4 (5), pp. 833-840.

Pasamontes, L., M. Haiker, M. Wyss, M. Tessier, A.P.G.M. van Loon (1997): Gene Cloning, Purification, and Characterization of a Heat-Stable Phytase from the Fungus *Aspergillus Fumigatus*. *Applied Environmental Microbiology* 63, pp. 1696-1700.

- de Pee, S., C.E. West, D. Permaesih, S. Martuti, Muhilal, and J.G. Hautvast (1998): Orange Fruit Is More Effective Than Are Dark-Green, Leafy Vegetables in Increasing Serum Concentrations of Retinol and b-Carotene in Schoolchildren in Indonesia. *American Journal of Clinical Nutrition* 68, pp. 1058-1067.
- Pearson, J.N., and Z. Rengel (1995): Uptake and Distribution of Zn and Mn in Wheat Grown at Sufficient and Deficient Levels of Zn and Mn During Growth Development. *Journal of Experimental Biology* 46, pp. 841-845.
- Ponnamperuma, F.N. (1982): Genotypic Adaptability as a Substitute for Amendments on Toxic and Nutrient-Deficient Soils. In: A. Seife (ed.). *Plant Nutrition 1982*. CAB, Slough, UK, pp. 467-473.
- Raboy, V. (1996): Cereal Low Phytic Mutants: A 'Global' Approach to Improving Mineral Nutritional Quality. *Micronutrients and Agriculture*, No. 2, Federation of American Scientists, Washington, D.C. Rahman, S., G.F. Vance, and L.C. Munn (1993): Salinity Induced Effects on the Nutrient Status of Soil, Corn Leaves, and Kernels. *Community in Soil Science and Plant Analysis* 24, pp. 17-18.
- Rengel, Z., and R.D. Graham (1995): Importance of Seed Zn Content for Wheat Growth on Zn-Deficient Soil, I; Vegetative Growth. *Plant and Soil* 173, pp. 259- 266.
- Rengel, Z., and R.D. Graham (1995): Importance of Seed Zn Content for Wheat Growth on Zn-Deficient Soil, II; Grain Yield. *Plant and Soil* 173, pp. 267-274.
- Schledz, M., S. Al-Babili, J. Lintig, H. Haubruck, S. Rabbani, H. Kleinig, and P. Beyer (1996): Phytoene Synthase from *Narcissus Pseudonarcissus*: Functional Expression, Galactolipid Requirement, Topological Distribution in Chromoplasts and Induction During Flowering. *Plant Journal* 10 (5), pp. 781-792.
- 25 Senadhira, D., and R.D. Graham (1999): Genetic Variation in Iron and Zinc Concentrations in Brown Rice. *Micronutrients in Agriculture* 3, pp. 10-12.
- Sillanpaa, M. (1990): Micronutrient Assessment at the Country Level: An International Study. *FAO Soils Bulletin* 48, Food and Agriculture Organization of the United States, Rome. Skikne, B., D. Fonzo, S.R. Lynch, and J.D. Cook (1997): Bovine Ferritin Bioavailability in Man. *European Journal of Clinical Investigations* 27, pp. 228- 233.
- Smith, J.C. (1996): The Vitamin A - Zinc Connection. *Micronutrients and Agriculture*, No. 1, Federation of American Scientists, Washington, D.C. Sparrow, D.H., and R.D. Graham (1988): Susceptibility of Zinc-Deficient Wheat Plants to Colonization by *Fusarium Graminearum* Schw. Group 1. *Plant Soil* 112, pp. 261-266.
- Taylor, P.G., C. Martinez-Torres, E.L. Romano, and M. Layrisse (1986): The Effect of Cysteine-Containing Peptides Released During Meat Digestion on Iron Absorption in Humans. *American Journal of Clinical Nutrition* 43, pp. 68-71.
- Thongbai, P., R.J. Hannam, R.D. Graham, and M.J. Webb (1993): Zinc Nutrition and

Rhizoctoria Root Rot of Cereals. *Plant Soil* 153, pp. 207-214.

Welch, R.M. (1986): Effects of Nutrient Deficiencies on Seed Production and Quality. *Advances in Plant Nutrition* 2, pp. 205-247.

Welch, R.M. (1993): Zinc Concentrations and Forms in Plants for Humans and Animals. In: A.D. Robson (ed.). *Zinc in Soils and Plants*. Kluwer Academic Publishers, Boston, pp. 183-195.

Welch, R.M. (1996): The Optimal Breeding Strategy Is to Increase the Density of Promoter Compounds and Micronutrient Minerals in Seeds; Caution Should Be Used in Reducing Anti-Nutrients in Staple Food Crops. *Micronutrients and Agriculture*, No. 1, Federation of American Scientists, Washington, D.C. Welch, R.M., W. House, S. Beebe, D. Senadhira, G. Gregorio, and Z. Cheng (1999): Testing Iron and Zinc Bioavailability in Genetically Enriched Bean (*Phaseolus Vulgaris* L.) And Rice (*Oryza Sativa* L.) Using a Rat Model. Paper presented at the workshop "Improving Human Nutrition Through Agriculture: The Role of International Agricultural Research," 5-7 October, Los Baños, Philippines. Welch, R.M., M.E. Smith, D.R. van Campen, and S.C. Schaefer (1993): Improving the Mineral Reserves and Protein Quality of Maize Kernels Using Unique Genes. *Plant and Soil* 171, pp. 155-156.

Wise A. (1995): Phytate and Zinc Bioavailability. *International Journal of Food Science and Nutrition* 46, pp. 53-63.

World Bank (1994): *Enriching Lives: Overcoming Vitamin and Mineral Malnutrition in Developing Countries*. Development in Practice Series, World Bank, Washington, D.C. Ye, X., S. Al-Babili, A. Klöti, J. Zhang, P. Lucca, P. Beyer, I. Potrykus (2000): Engineering the Pro-Vitamin A (b-Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm. *Science* 287, pp. 303-305.