2. Genetically modified food crops and their contribution to human nutrition and food quality

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2.1. Introduction

This chapter discusses the potential of biotechnology to improve the health and nutrition of consumers in developing countries. In the relatively wealthy countries of Europe, North America and elsewhere, consumers spend perhaps 10\% of their income on food. For the most part consumers in developed countries are free of classical nutrient deficiencies, although over-consumption is a problem for some. Also in relatively wealthy countries there is, in general, good access to affordable medical care to meet health needs and most consumers in rich countries have access to a relatively inexpensive supply of safe and healthy food. In these settings, the possibility that biotechnology might reduce the price of food or make food more beneficial to health is a relatively minor concern. Rather, public debate about genetically modified foods (GMFs) appears to have focused on the potential for harm to either the environment or health without a clear definition of benefit to the consumer.

The situation, of course, is quite different in poor countries where malnutrition and ill health are frequent. Poor consumers typically spend 70\% of their incomes on food, and diets consist primarily of staple foods, which lack the vitamins, minerals and, very likely, other food components necessary to sustain good health and minimise the risk of adult onset diet-related chronic diseases. In addition, low incomes typically preclude the poor from access to adequate health care.

There are three broad ways that biotechnology may benefit consumers in developing countries. First, biotechnology offers a powerful, new tool to improve crop productivity, both by making conventional breeding faster and more efficient and, more controversially, by the insertion of novel genes in a crop species, by use of transgenic methods. This includes the ability to bring new lands with unfavourable growing environments, such as those with high saline soils, under production. Second, pesticide applications may be reduced through adoption of \textit{Bt}-containing crops (i.e. crops with insect resistance owing to the introduction of the \textit{Bacillus thuringiensis} (\textit{Bt}) gene encoding an insect-specific toxin). Reducing pesticide use can improve the health of farmers, in addition to lowering input costs. Third, transgenic methods may be used to improve the micronutrient content and/or bioavailability of commonly eaten foods in developing countries.

The first pathway, that is improving crop productivity, is associated with an often-asked question—Does feeding the world depend on the use of biotechnology?—The answer largely depends on perceptions of the
current situation. Countries around the world are already using most of the arable land available for agriculture. As populations continue to grow, increasing the supply of food to meet future world food needs will depend on increasing yields. This, in turn, requires investments in agricultural research to increase the yield potentials of specific food crops as well as fish and livestock production.

Conventional breeding of cereals has been especially successful over the past three decades. Through research and extension efforts associated with the ‘Green Revolution’, the rate of increase of production of rice, wheat, and maize in developing countries has outpaced population growth and other factors associated with increased demand (for example higher household incomes), such that the inflation-adjusted prices of rice and wheat fell by 30–40% between 1970 and 1997 (World Bank, 1990–1999).

World supplies and prices of cereals are inter-connected through international trade. Advances in conventional breeding in Australia, Europe, and North America have also contributed to falling cereal prices. Continued low prices of cereals are likely as long as investments in agricultural research continue, although a worrisome trend is that funds for public research in agriculture have been declining (Rosegrant, Paisner, Meijer, & Witcover, 2001).

It is often said that there is enough food in the world to feed everyone. Except for some pockets of extreme poverty and dislocations owing to natural disasters and civil strife, this is largely the case for cereals. Cereals (and roots and tubers) provide the cheapest sources of energy to meet requirements for poor populations. The availability of affordable staple foods prevents explicit hunger, as defined narrowly in terms of energy requirements.

However, a range of non-staple foods, such as animal and fish products, fruits, pulses, and vegetables, are foods rich in bioavailable vitamins and minerals or micronutrients and possibly other food components, which are necessary for good health and a productive life. As discussed in more detail below, micronutrient undernutrition is widespread in poor countries, affecting more than one-half of the population in the developing world. This situation is due primarily to diets of low nutritional quality. The poor want to eat larger amounts of non-staple foods, and most often these foods are available in local markets. However, such non-staple foods are simply too expensive to be eaten by the poor in large quantities owing to a combination of high prices and low purchasing power. The inflation-adjusted prices of non-staple foods have not been declining; in some cases they have been increasing over the past 30 years.

Because of poor dietary quality and consequent micronutrient malnutrition, some people, especially children and their mothers who have higher requirements for vitamins and minerals, have higher mortality, become sick more often, have their cognitive abilities compromised for a lifetime, and are less productive members of the workforce than might otherwise have been the case. Their quality of life and aggregate economic growth are unnecessarily compromised. To make matters worse, the adverse synergy between undernutrition and infection results in nutrition requirements above those that characterise the needs of well-nourished populations.

In developing countries demand for non-staple foods is growing faster than demand for cereals, as ever-richer households purchase more non-staple foods than cereals, with consequent pressure on non-staple food prices to increase. In addition, increasing the productivity of non-staple foods through agricultural research (and so lowering prices) is much more expensive than increasing the productivity of staple foods, because of the far larger number of non-staple foods involved.

From this perspective, then, the world is already failing to provide an adequate supply of non-staple foods for a large proportion of its population, and prospects are somewhat bleak for short- to medium-term improvements. Biotechnology affords a powerful new tool to help increase the supply of food produced in the world and redress, in part, situations characterised by poor dietary quality.

Chapter 3 discusses ways, including reduced use of pesticides (i.e. the second pathway, above), in which biotechnology can provide breakthroughs in improving the productivity of crops. However, it is well beyond the scope of this special issue to speculate on the rate of growth of world food supplies with and without the use of biotechnology.

A third pathway through which biotechnology can improve the nutrition and health of consumers in developing countries is through increasing the vitamin and mineral content and bioavailability of staples and other foods and/or reducing allergens, an approach which is focused on in this chapter. It must be emphasised that none of the products discussed in this chapter has been introduced commercially, though they are in various stages of research and development. Thus, importantly, both the efficacy and effectiveness of this pathway requires documentation.

Although it is obviously important to provide an account of the range of specific efforts being developed to improve the nutritional content and health-enhancing properties of particular foods, it is also necessary to place these activities in the context of alternative interventions (i.e. interventions that do not involve the use of
biotechnology) that might solve these malnutrition and health problems in other ways. This is accomplished by (i) examining the relative costs of nutrient supplementation and commercial nutrient fortification programs that are already being implemented to reduce micronutrient malnutrition and (ii) examining the recent history of attempts to improve the micronutrient content of staple food crops—efforts that have involved use both of biotechnology and conventional plant breeding. Recent developments in efforts to breed iron- and beta-carotene-dense rices are described in this chapter as a concrete example, illustrating several key generic issues associated with the use of biotechnology to breed for characteristics that have direct benefits for consumers.

It is important to keep in mind that the final, sustainable solution to micronutrient malnutrition in developing countries is a substantial improvement in dietary quality by higher consumption of pulses, fruits, vegetables, fish, and animal products, which the poor need and already desire, but cannot presently afford. Realisation of this goal of high quality diets will require (i) substantial investments by farmers, private businesses and public agencies to build the infrastructure to produce and bring to market the requisite supply of non-staple foods, (ii) sound government policies to stimulate agricultural and economic growth, (iii) considerable increases in the incomes of the poor during the course of economic development over many decades, or (iv) other strategies (e.g. subsidies) designed to enhance the accessibility of these products to wider populations. Meanwhile breeding staple foods that are dense in minerals and vitamins can provide a low-cost, sustainable, although as yet unproven strategy for reducing existing levels of specific micronutrient undernutrition.

Sections 2.2–2.5 (below) discuss, in some detail, iron- and beta-carotene-dense rice, as an example of the development of a GMF crop of potential importance in developing countries. Section 2.6 describes the potential of transgene technologies to enhance nutritional and quality factors of food crops in general; this is important in both developing and developed countries, though perhaps has more immediate relevance in the latter.

2.2. Potential contribution of GM technology: a case study on breeding of iron- and beta-carotene-dense rices to help alleviate malnutrition

2.2.1. Background to the problem of micronutrient malnutrition in poor countries, its economic costs and costs of present interventions to address the problem

It is only relatively recently that nutritionists working in developing countries have demonstrated conclusively that, during times of relative economic and political stability, many children and adults, particularly women in their child-bearing years, suffer more from a lack of essential vitamins and minerals in their diets than from a lack of calories. People, for the most part, are not aware that their diets lack such trace nutrients and so do not associate these deficiencies with listlessness, poor eyesight, impaired cognitive development and physical growth, more frequent and severe bouts of illness, and high mortality. For this reason, the general problem of poor dietary quality has been dubbed ‘hidden hunger’.

It is useful to summarise very briefly the scale and consequences of micronutrient malnutrition in developing countries. It is estimated that over 3 billion people in developing countries are iron-deficient (Administrative Committee on Co-ordination, Subcommittee on Nutrition, & International Food Policy Research Institute, 2000). The problem for women and children is more severe because of their greater physiological need for iron. In poor countries, more than half of pregnant women and more than 40% of non-pregnant women and preschool children are anaemic. Iron deficiencies during childhood impair physical growth, mental development and learning capacity. In adults, iron deficiency reduces the capacity to do physical labour. Severe iron deficiency is a leading contributor to death among women during childbirth, owing to low haemoglobin levels in the light of physiological blood loss.

Globally, about 3 million children of preschool age have visible eye damage owing to a vitamin A deficiency. Annually, an estimated 250 000–500 000 preschool children go blind from this deficiency and about two-thirds of these children die within months of going blind. Estimates of the subclinical prevalence of vitamin A deficiency range from 100 to 250 million. A number of clinical trials in developing countries have shown that vitamin A supplementation, as capsules distributed biannually in high doses, as weekly distributed low doses (consistent with levels attainable through food) or as a fortified product in diets can reduce mortality rates among preschool children on average by 23% (Beaton et al., 1993).

Deficiencies in several other micronutrients, in particular zinc, may also be widespread, with equally serious consequences for health. However, because for some of these micronutrients, specific indicators are lacking to screen for deficiencies, they have not received as much attention as iron, vitamin A and iodine deficiencies.

The World Bank (1994) estimates that at the levels of micronutrient malnutrition existing in South Asia, 5% of gross national product is lost each year due to deficiencies in the intakes of just three nutrients, namely iron, vitamin A and iodine. For each 50 million in
population, this translates into an economic loss of US $1 billion per year.

Some general cost calculations can be made for interventions to relieve iron and vitamin A deficiencies by supplementation and fortification in South Asia, which has a total population of roughly 1.25 billion people. The costs of vitamin A pills themselves are low: an often-quoted cost of vitamin A supplementation, which includes the costs of delivery, is US $0.50 per person per year (i.e. US $0.25 per capsule; World Bank, 1994). If one in every 12.5 people in South Asia were to receive supplements (i.e. 100 million people in total), this would cost US $50 million per year, or US $500 million over 10 years. An often-quoted cost of iron fortification is US $0.12 per person per year (World Bank, 1994). If a particular food vehicle fortified with iron were to reach 33% of the total (but untargeted) population in South Asia (i.e. 412 million people), the total cost would, again, be US $50 million annually, or US $500 million over 10 years. In absolute terms, these nutrient-specific interventions may seem to be large amounts of money, but they are very worthwhile investments and actually represent quite small percentages of the total economic activity of the South Asian economies.

The Micronutrient Initiative (MI)\(^2\) estimates that 1 billion vitamin A pills have been distributed worldwide since the inception of vitamin A distribution programmes up to the end of 2000 and that 500 million supplements are now given out annually.

A conservative estimate of the annual, recurrent cost of global vitamin A supplementation programmes is, therefore, US $125 million. Assuming that 75% of the world’s need for vitamin A is met, recurrent annual estimated costs to meet all worldwide needs would be about US $165 million, or $1.65 billion over a 10-year period.

Although benefit–cost ratios are quite high, supplementation and fortification programmes must be sustained at more or less the same level of funding year after year in any given country. If investments are not sustained, benefits of course disappear.

Investments in plant breeding research and dissemination are far lower (see Section 2.3.2) and potentially long lasting. Benefits of agricultural research at a central location can be leveraged throughout the world and across time. Breeding for staple crops with high bioavailable micronutrient content in their seeds, hereinafter referred to as ‘biofortification’, treats the underlying cause of micronutrient undernutrition. Although plant breeding can involve relatively long lead times of 8–10 years before nutritious varieties can be developed and their adoption by farmers can be initiated, such a strategy is sustainable once breeding has been completed, and seeds have been disseminated and adopted by farmers. Biofortification has the potential to provide coverage for remote rural populations, which supplementation and fortification programs may not reach, and it inherently targets the poor who consume high levels of staple foods and little else.

However, efficacy and effectiveness trials and other studies have yet to be undertaken to determine both the degree to which nutritionally dense varieties can improve micronutrient status and whether there are unintended negative effects. For example, untargeted approaches designed to increase bioavailable dietary iron substantially must be considered with care. The documented risks of iron deficiency must be weighed against the potential for genetically determined increased risks of toxicity from iron overload, given the relatively common occurrence of heterozygosity for haemachromatosis. Thus ‘permanent’ changes in the food supply, which result in long term ‘high’ intakes of iron should be considered in the light of the incompletely described consequences of relevant polymorphisms in genes that appear to play roles in the regulation of iron metabolism. Such risks have been studied most adequately in developed countries (Merryweather-Clarke, Pointon, Sherman, & Robson, 1997).

However, according to an expert group convened to consider micronutrient supplementation and fortification with iron, iron overload disorders and haemachromatosis are rare, even in those populations of European origin most susceptible to them; therefore, the use of fortification and supplementation as public health interventions for preventing and controlling iron deficiency should not be constrained (UNICEF/UNU/WHO/MI, 1999). It is safe to assume that the same conclusions would apply to biofortification. If biofortification eventually were to replace supplementation in some areas, there is the possibility that biofortification might lessen risks of overdosing, such as that recently reported in India, where toxicity associated with a mass distribution of a vitamin A concentrate to children was thought, possibly, to have been the result of a changed delivery routine (Sharma, 2001).

2.2.2. Improving the nutrient content and bioavailability of minerals and vitamins in staple food crops through biofortification

Breeding strategies

There are three breeding sub-strategies that may be applied individually or in various combinations. These are (i) reducing the level of antinutrients in food staples,
which inhibit the bioavailability of minerals and vitamins, (ii) increasing the levels of nutrients and compounds that promote the bioavailability of minerals and vitamins, and (iii) increasing the mineral and vitamin content. Experience with increasing the mineral and vitamin content of rice is discussed in some detail in Section 2.2.3.

Reducing antinutrients

A breeding strategy of lowering the level of antinutrients, such as phytic acid, which binds metals in grain, has often been suggested as a way to increase the bioavailability of minerals already consumed.

Phytin is the primary storage form of phosphorus in most mature seeds and grains and is an important compound required for early seed germination and seedling growth (Welch, 1986; Wise, 1995). Phytin plays an important role in determining mineral reserves of seeds and thus contributes to the viability and vigour of the seedling produced (Welch, 1993).

Graham and Welch (1996) argue that selecting for seed and grain crops with substantially lower phytin content could have an unacceptable effect on production, especially in regions of the world where there are soils of low phosphorus status and/or poor micronutrient fertility. Such attempts to lower the antinutrient content of seeds and grains significantly require a major shift in seed or grain composition. Because most of the antinutrients known to occur in seeds and grains are major organic constituents of these organs, they may play beneficial roles in plant growth and may have other unidentified roles in human health. Therefore, Graham and Welch argue against a breeding strategy that attempts to increase iron bioavailability by reducing antinutrient content. Antinutrients may also reduce absorption of potentially toxic trace minerals such as cadmium and other heavy metals (McLaughlin, Parker, & Clarke, 1999).

However, Raboy (1996) 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% 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 mutants are presently being tested for agronomic performance and effects on micronutrient status in humans.

A related strategy is to increase the level of phytase. Phytase can break down phytates, thus increasing bioavailability of minerals. Phytases have been used successfully in animal feeds (Nelson, Shieh, Wodzinski, & Ware, 1971; Rimbach & Pallauf, 1993; Yi, Kornegay, & Veit, 1996). Phytase is present in the endosperm of rice, for example, but is destroyed when rice is boiled for eating. Heat-stable phytase may be introduced in food staples through genetic transformation (Holm, Kristiansen, & Pedersen, 2002).

Increasing levels of compounds that improve absorption and utilisation of nutrients

Certain amino acids, such as cysteine, 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 positive effects on iron and/or 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 antinutrients on iron and zinc bioavailability. These amino acids are normal constituents of plants, so relatively small increases in their concentrations in plant tissues should not have adverse consequences on plant growth (Graham & Welch, 1996).

However, when manipulating amino acid content, the net effects on the resultant product’s protein quality should also be considered. The three most common measures of protein quality are protein efficiency ratios, biological value and net protein utilisation. These measures are expressed as quotients of retained nitrogen (a constituent of protein) divided by the total intake of protein nitrogen. Among the determinants of protein quality are amino acid pattern, absolute levels of protein intake and physiological requirements for essential amino acids. Essential amino acids are those that must be provided pre-formed because of an inability for their de novo synthesis. The higher the quality of a specific protein, the more efficiently it is utilised and the less is needed to meet protein requirements. Thus changes in protein content or amino acid pattern of the resultant protein mix can have significant effects on the efficiency of protein utilisation to meet nutritional requirements.

An additional possibility is that as intakes of beta-carotene (converted to vitamin A after ingestion) are increased, the utilisation of absorbed iron may be promoted and vice-versa. That is, there are possible synergies between increasing intakes of these two nutrients.

3 When such mutants are used as animal feeds, this also avoids what has become a serious pollution problem—excretion of utilised phytic acid.
There is already considerable evidence for synergism between vitamin A and zinc intakes (Smith, 1996).

**Feasibility**

*Will famers adopt nutrient-rich varieties?* Impact on plant nutrition is a particular concern of agriculturalists, whose first priority may be to raise the yield potential of plants rather than to address human nutrition problems. If yield and profitability are compromised by breeding for nutrient content, farmers will not want to adopt nutritionally enhanced cultivars. However, relatively recent research has shown that mineral-dense seeds assist plants in resisting disease and other environmental stresses (Graham, Welch, & Bouis, 2001; Welch & Graham, 1999). More seedlings survive and initial growth is more rapid. Ultimately, yields are higher, particularly in trace mineral ‘deficient’ soils in arid regions.4

*Will breeding for micronutrient-dense seeds change the processing or consumer characteristics of staple foods?* Mineral micronutrients comprise a tiny fraction of the physical mass of a seed; for example, 5–10 mg/kg (ppm) in milled rice, and as much as 100 mg/kg in micronutrient-dense bean seeds. It is not known whether such small amounts will alter the appearance, taste, texture, or cooking quality of foods. The dissemination strategy for trace minerals, then, would be to include the mineral-density trait in as many varietal releases as possible, in order to benefit a high proportion of the population, without having to rely on behavioural change as a condition for success. This would be much the same approach as adding fluoride to drinking water in developed countries. As with the example of fluoride, information on increased nutrient content would be made publicly available.

However, increasing levels of beta-carotene will turn varieties from white or light colours of yellow to dark yellow and orange. Often white varieties are much preferred by consumers (e.g. milled rice, wheat flour, maize, cassava). Major nutrition education programs will probably be necessary to encourage consumers to switch to more nutritious varieties. However, if these nutrition education programs are successful, the yellow-orange colour will mark the more nutritious varieties from the less nutritious, and a disadvantage will have been turned into an advantage (Hagenimana & Low, 2000).

2.2.3. Increasing micronutrient content in rice

**Conventional breeding**

In a survey of traditional and modern varieties of rice, Graham, Senadhira, Beebe, Iglesias, and Ortiz-Monasterio (1999) reported that iron density in unmilled rice varied from 7 to 24 mg/kg (ppm) and zinc density from 16 to 58 mg/kg. Nearly all the widely grown green revolution varieties were similar, at about 12 and 22 mg/kg for iron and zinc, respectively. The best lines discovered in the survey of the existing germplasm collection were, therefore, twice as high in iron and 1.5 times as high in zinc as the most widely grown varieties. These ratios, therefore, are benchmarks for iron and zinc density, which seem to be reasonably attainable using conventional breeding.

High iron and to a lesser extent high zinc concentrations were subsequently shown to be linked to the trait of aromaticity. Most aromatic rices, such as jasmine and basmati types, are high in both iron and zinc, and generally in most minerals (Graham, Senadhira, & Ortiz-Monasterio, 1997; Graham et al., 1999; Senadhira & Graham, 1999). A promising aromatic variety, designated IR68-144, already being tested at the International Rice Research Institute (IRRI) owing to its superior agronomic and consumer characteristics, was found to be high in iron (Gregorio, 2002).

Expression of 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 (implying that the genotype-environment interaction is not serious), a finding that is sufficient to encourage a breeding effort. Environmental factors considered by one or more crop programs include acid soils, alkaline soils, saline soils, acid-sulphate soils, iron-deficient soils, time of planting, field site, season, nitrogen fertilisation, phosphorus fertilisation, potassium status, elevation and drought stress (Graham et al., 2001).

**Transgenic methods**

*Increasing iron.* 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 ferritin is used by both plants and animals as the storage form of iron (Theil, Burton, & Beard, 1997);
orally administered ferritin, in non-transgenic foods (Beard, Burton, & Theil, 1996), and in transgenically-derived ferritin-rich rice (Murray-Kolb et al., 2002) can provide a source of iron for treatment of anaemia in rats.

Human studies with extrinsically radiolabelled animal ferritin have indicated that, when added to a meal, iron from ferritin is only about half as well absorbed as iron from vegetables (Martinez-Torres, Renzi, & Layrisse, 1976; Taylor, Martinez-Torres, Romano, & Layrisse, 1986) and iron as ferrous sulphate (Skikne, 1976; Taylor, Martinez-Torres, Romano, & Layrisse, 1986) and iron as ferrous sulphate (Skikne, Fonzo, Lynch, & Cook, 1997). However, a recent study has shown that intrinsically radiolabelled iron in soybeans is highly bioavailable (27%) to iron-deficient women (Murray-Kolb, Welch, Theil, & Beard, 2003). About 50% of the iron in the soybeans was ferritin; the remaining iron, ferric phytate, is not thought to be highly bioavailable.

Goto, Yoshihara, Shigemoto, Toki, and Takaïwa (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 glutation promoter, to mediate the accumulation of iron specifically in the grain. The transgenic seeds stored up to three times more iron than normal seeds. Iron levels in the whole (unmilled) seeds of the transformants varied from 13 to 38 mg/kg (ppm); pooled mean values were 23 mg/kg for transformants and 11 mg/kg for non-transformants. The average iron content in the endosperm was 3.4 mg/kg in the transformant and 1.6 mg/kg in the non-transformant. 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. Trace minerals cannot be synthesised by plants and must be transported from the soil, through the plant and to the seed. Several processes and genes may be involved. The authors speculate that the amount of iron accumulation is restricted by transport of iron to the ferritin molecule, rather than simply by levels of ferritin protein. Thus, it may be possible to store larger amounts of iron in the ferritin molecule by co-integrating the ferritin gene and the iron reductase-like transporter gene. Robinson, Procter, Connolly, and Gueriniot (1999) report a newly discovered ferric-chelate reductase gene, which allows plants to absorb more iron from the soil, thus introducing the possibility of widening the scope of rice varieties with high iron uptake.

A doubling of the iron content in rice using a ferritin gene derived from Phaseolus vulgaris has been reported (Gura, 1999). Metallothioneine was also expressed in the rice grain, increasing the cysteine content seven-fold.

**Introducing a gene which codes for heat-stable phytase.** The level of phytase, which breaks down phytic acid, is normally low in milled rice. Several studies have already demonstrated the usefulness of adding phytase to the rice diets of poultry (e.g. Adrizal & Sell, 1996; Farrell & Martin, 1998; Martin, Nolan, Nitsan, & Farrell, 1998; Munaro, Lopez, Lopez, & Rutz, 1996; Teichman, Lopez, & Lopez, 1998). The phytase that does exist in rice seeds will hydrolyse the phytic acid present at lower pH levels, if seeds are left to soak until germination in water. However, boiling destroys the phytases that occur naturally in rice.

In one study, an amino acid was changed in the protein sequence to make the phytase heat stable (Pasmontes, Haiker, Wyss, Tessier, & Van Loon, 1997). The phytase was active under the pH conditions of digestion and degraded phytic acid in a very short time, during model in vitro digestion.

Lucca, Hurrell, and Potrykus (2001) reported the introduction into rice of a transgene for this particular heat-stable phytase from Aspergillus fumigatus; the transgene increased the level of phytase activity by 130-fold. Unfortunately after expressing the gene in the grain, the phytase was no longer stable to heat and lost its activity on boiling.

A new approach is now under investigation in which the A. fumigatus phytase gene would break down phytate during seed maturation [when pH levels (6–7) would be conducive to phytase activity]. Fusing the globulin promoter to the phytase gene, which tests have shown is expressed only in the endosperm of rice, which is consumed after milling, should prevent seed germination being compromised (after harvesting and replanting) owing to low phosphorus content. The hope is that the phosphorus in the outer layers of the seed (which is milled away for consumption but not for planting) would remain unaffected (Lucca, Hurrell, & Potrykus, 2002).

**Increasing levels of compounds that improve absorption and utilisation of nutrients.** Levels of lysine, which is an important, essential and limiting amino acid in rice and which might promote the uptake of trace minerals, can be improved by transgenic methods (Datta & Bouis, 2000). Introduction of two bacterial genes, expressing DHSPS (dihydrodipicolinic acid synthase) and AK (aspartokinase) enzymes, encoded by the Corynebacterium dapaA gene and a mutant Escherichia coli lysiC gene, has enhanced lysine levels about five-fold in canola and soybean seeds (Falco et al., 1995).

**Adding beta-carotene.** Beta-carotene, a precursor of vitamin A, does not occur naturally in the endosperm of rice. Ye et al. (2000) have reported generating transgenic plants that produce grain with yellow-coloured endo-
sperm. Biochemical analysis confirmed that the colour represents beta-carotene (provitamin A). The reported level of beta-carotene in one gram of the dry, uncooked transformed rice (Golden Rice) is 1.6 μg. Research is presently underway to increase the level of beta-carotene in transformed rice, with promising ‘proof of concept’ results in model systems (Beyer & Potrykus, 2001).

According to Ye et al. (2000), psy (cloned from Narcissus pseudonarcissus; Schledz et al., 1996), cry1 (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 subunit (Misawa et al., 1993). The insertion of a multiple gene cassette is a remarkable accomplishment, considering that most traits engineered to date have only required the addition of a single gene (Guerinot, 2000).

A discussion on the degree to which vitamin A intakes may be improved by the use of Golden Rice in the rural Bangladesh context is provided below (Section 2.3.1).

2.3. Outcomes and impacts of breeding for iron- and beta-carotene-dense rice

2.3.1. Impact on iron- and beta-carotene intakes

For poor populations, food staple consumption so dominates diets that primary food staples provide in the range of 40–55% of total iron intakes (Bouis, Graham, & Welch, 2000). If a single food staple provides 50% 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 result in a 50% increase in total iron intakes. It would seem evident that a 50% increase in intakes of iron, if it were to be as bioavailable as iron already in the diet, would be of considerable benefit to anaemic women with such low iron intakes. Some empirical evidence is available from a recently published study of a population of Bangladeshi rural women (Bhargava, Bouis, & Scrimshaw, 2001). The study measured the relationship between diets and blood haemoglobin. The estimated relationships suggested that a 50% increase in iron intakes from biofortified rice would reduce rates of anaemia among these women by a minimum of 3% (e.g. from 53–50%). This is an estimate of the percentage of women who would cross above the threshold of 12 g/dl haemoglobin as a result of consuming biofortified rice. Women who remained below this threshold presumably would also derive some benefit from a 50% increase in iron intakes.

Biofortified varieties may reach undernourished people for a cost of only US $0.02–0.03 per person per year (Hunt, 2002), compared with a cost of US $0.12 for commercial iron fortification (see Section 2.2.1) and costs exceeding US $3.00 per person for iron supplements (World Bank, 1994). In the study by Bhargava et al. (2001), the effect of iron supplements in reducing anaemia levels was estimated to be three times as strong as that of biofortified rice. Given that, per person reached, the cost of iron supplements is more than 100 times the cost of biofortified rice (i.e. US $3.00 compared with < US 0.03 cents), then the cost per annual case of anaemia avoided is more than 30 times higher for supplements than for biofortified rice.

For the same rural Bangladeshi population referred to above, it is also possible to estimate the percentage increase in total provitamin A intake provided by replacement of presently eaten varieties of rice with Golden Rice (1.6 μg beta-carotene per gram of dry rice endosperm; Ye et al., 2000). For this poor population, animal and fish intakes provide only 3% of total energy (Bouis et al., 1998). Intakes of preformed vitamin A are, therefore, negligible and vegetables provide greater than 90% of total provitamin A intakes. The US Institute of Medicine recommends that a ratio of 12 to 1 be used for converting beta-carotene to retinol activity equivalents when evaluating mixed diets for healthy populations (Institute of Medicine, Food and Nutrition Board, 2001). Estimated average intakes for Bangladeshi women and for preschool children (2–5 years) are 260 and 106 retinol equivalents, respectively. Recommended dietary allowances (RDAs) for healthy women and children (4–8 years) are 700 and 400 retinol equivalents, respectively (Institute of Medicine, Food and Nutrition Board, 2001). Vitamin A intakes in Bangladesh are, therefore, currently well below recommended intakes.

If Golden Rice were to replace the non-transgenic rice, total provitamin A intakes would be increased by 25% for adult women and preschool children; using a ratio of 12 to 1 to convert provitamin A in both vegetables and Golden Rice to retinol activity equivalents, total vitamin A intakes would still remain well below requirements.

However, the conversion rate from beta-carotene to retinol activity equivalents is a function of the food matrix in which the beta-carotene is present, the form in

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5 Due to measurement error in a number of difficult-to-record variables, this estimate is likely to be a considerable underestimate. Roughly, it can be estimated that each additional 1 mg of non-haeme iron added to rice (or to any other food in the diet) would reduce the anaemia prevalence by 1%. To see that this is a lower bound estimate, it should be considered that, at this rate, an additional 36 mg of iron, or twice the recommended daily allowance for women, would be required to lower prevalence by 36% e.g. from 50 to 14%.

which the food is prepared, the presence of dietary fat, and other factors. For example, the conversion rate for some leafy, green vegetables has been estimated to be 26 to 1, whereas that from some orange fruits may be 13–15 to 1 (de Pee et al., 1998). It may be that the beta-carotene in the rice endosperm matrix would allow a relatively high conversion rate. Rice is a very simple food matrix, which is totally digestible. Therefore, carotenoids may be more easily released from rice than from an undigested matrix of vegetable origin (Tyssandier, Lyan, & Borel, 2001).

It is also important to note that RDAs are set at levels to maintain 4-month body stores. About one-half of the RDA is needed to prevent vitamin A deficiency (Institute of Medicine, Food and Nutrition Board, 2001). For the Bangladeshi population described above, adult women would reach one-half of the RDA (deficiency prevention) at an 8 to 1 conversion ratio and preschool children at a 4 to 1 conversion ratio. However, such a calculation assumes that only Golden Rice would be consumed (complete substitution with other rice varieties) and that no beta-carotene would be lost in processing and cooking. Research is currently being undertaken to increase the beta-carotene content of new lines of Golden Rice (Beyer et al., 2002).

However, the conversion factors and RDA, assume a healthy population, which is unlikely for poorer segments of the Bangladeshi population, or for poorer populations in other less developed countries. Undernutrition is prevalent and enteric infections frequent; this erodes the integrity of the intestinal absorption surface where the mucosal beta-carotene cleavage enzyme normally resides. A vitamin A deficient state may partially compensate by increasing both the conversion efficiency of the beta-carotene that is absorbed and its efficient utilisation. Data on how these numerous variables interact to influence absorption efficiency from Golden Rice are limited. Therefore, uncertainty surrounds estimates of the contribution to vitamin A status that high beta-carotene staple foods can make among poor populations in developing countries, whether such foods are selected from existing gene banks or developed using classical breeding techniques or transgenic methods. Efficacy trials will help to reduce some of this uncertainty.

2.3.2. Costs and benefits of a biofortification strategy

Development of iron- and zinc-dense varieties of rice or wheat using conventional breeding might cost as much as US $10 million each over 10 years, including the costs of nutrition safety and efficacy tests, the costs of dissemination in selected regions, and the costs of an evaluation of nutritional and economic impact (Hunt, 2002). As indicated above (Section 2.2.1), such an investment (of US $20 million in two crops, rice and wheat) is projected to have far reaching impacts if efficacy and effectiveness are achieved. Under such conditions, it would be reasonable to extrapolate benefits to countries around the world, as countries would need only to invest in adaptive breeding and dissemination costs. Moreover, benefits should be sustainable at low maintenance costs. Benefits from breeding advances typically do not disappear after initial investments and research have been successful, as long as an effective domestic agricultural research infrastructure is maintained.

A simulation model, which was developed for Bangladesh and India, based on development of iron- and zinc-dense varieties of rice and wheat, which were assumed to be adopted on only 10% of approximately 83 million hectares planted to rice and wheat, supported the expectation of enormous economic benefits from a biofortification strategy (Hunt, 2002). Total costs were estimated to be US $42 million which, in addition to the US $20 million in central development costs for rice and wheat, included costs of adaptive and maintenance breeding and extension costs. These conservative assumptions suggest that the returns that come on-stream during the second decade of research and development would be about $1.2 billion in benefits from better nutrition.

A more formal economic evaluation of biofortification in Bangladesh and India, in which the ratio of the present value of benefits to the present value of costs was discounted at a 3% rate (commonly used for evaluation of social benefits), gave a benefit-cost ratio of 19, for returns, simply for better iron nutrition in humans; a similar ratio was found by Horton and Ross (1998) for fortification in South Asia. A different way of expressing the concept of discounting over time is the internal rate of return, in which the interest rate is calculated at which benefits would equal costs plus interest, if the funds were to be borrowed to make the investment. In this case the internal rate of return would be 29%, measured as benefits to human nutrition (Hunt, 2002).

In the longer term (years 11–25 of the simulation model) it was estimated that a total of 44 million cases of anaemia would be prevented if nutritionally improved varieties were to be adopted on 10% of rice and wheat areas in Bangladesh and India. This was based on a conservative assumption of only a 3% reduction in anaemia among those consuming the high-iron rice. Thus, costs would be about US $1 per case of anaemia per year prevented and US $0.03 per person per year for those whose iron intakes would be increased by 50% through consumption of iron-dense rice and wheat.

These estimates have been made for conventional breeding. It is not known whether transgenic methods would be more or less costly than conventional breeding methods, in part because the higher costs of meeting
stricter regulatory procedures for transgenic crops are not yet known. These higher regulatory costs for transgenic methods may be offset by the greater costs and time of backcrossing involved with conventional techniques. However, the relative costs of transgenic and conventional methods are of secondary importance, in the sense that, even if total costs for either method doubled the assumed costs for conventional breeding of $10 million over 10 years, biofortification would still be a very cost-effective proposition. For example, the very conservative benefit calculations above included only benefits for India and Bangladesh, while the varieties developed under full development cost assumptions could be used in other countries around the world as well. It is very difficult to predict benefits and breeding costs ex ante, so that it is important to make conservative estimates of benefits. Extrapolations are made on the assumption of specified levels of efficacy and effectiveness for interventions characterised by uncertainties that result from incomplete knowledge.

There is no a priori reason to believe that research costs will be higher or lower for a widely grown crop (e.g. for a cereal such as rice) than for a ‘minor’ crop (e.g. pulses). Given constant costs, benefits in agricultural research will always be higher for the more widely grown crop. Thus, there is bias in research investments toward those crops and, consequently, minor crops lag behind in terms of productivity increases. If the costs of developing transgenic crops are high owing to the need to meet regulatory procedures, the tendency to undertake research only on the more widely grown crop will increase. On the other hand, if biotechnology can lower the costs of undertaking agricultural research by making the process more efficient, this tendency will be mitigated.

2.4. Evaluating outcomes and impacts of iron- and beta-carotene-dense rice

2.4.1. Magnitudes of benefits and risks

Methods for evaluating the outcome and impact of nutritionally improved staple food crops fall into two categories:

- simple calculations of probable increases in total nutrient intakes from diets, such as those undertaken in section 2.3.1 in relation to RDAs; and
- assessment of the impact of the increased mineral and vitamin intakes on nutritional status.

The second category includes, in increasing order of precision, relevance, and cost:

- in vitro techniques (e.g. Glahn, Lee, & Miller, 1999);
- animal models (e.g. Welch et al., 2000);
- test meals;
- efficacy trials (e.g. Haas, del Mundo, & Beard, 2000); and
- effectiveness studies

2.4.2. Assessing likely beneficiaries of transgenic technologies

In vitro techniques and animal studies are relatively inexpensive and quick to implement and are primarily useful for screening candidate lines in a breeding program for relative bioavailability; however, they provide little information on the ultimate impact on micronutrient status of human populations of specific genotypes of crops being tested. For this assessment, several factors that affect the absorption and utilisation of nutrients must be evaluated. Such factors include:

- the micronutrient and health status of subjects; for example, those who are iron-deficient generally will absorb a higher percentage of iron in the diet;
- the level of compounds and nutrients in the meals being consumed that promote or inhibit the absorption of nutrients; for example, phytates inhibit the absorption of zinc; the presence of parasites; and
- the absence of other significantly limiting nutrients in the diets.

The relevance of results from test meals and efficacy trials, therefore, depend on how representative of the economically most disadvantaged population groups, are the nutritional status and diets of the test subjects. Efficacy trials, but not test meals, can allow for longer-term adaptations to the introduction of new foods and nutrients. Finally, effectiveness trials allow outcome and impact evaluation under usual community living situations.

An efficacy trial is currently being conducted with iron-dense rice strains developed using classical breeding techniques. A specific line of Golden Rice with stable and relatively high levels of beta-carotene is currently being selected for test meal evaluation of bioefficacy in humans. Several months will be needed to grow a sufficient quantity of rice with stable-isotope-labelled beta-carotene to undertake the study. Meanwhile experiments continue to develop improved lines with higher
levels of beta-carotene and even lines that produce retinol (Beyer & Potrykus, 2001; Beyer et al., 2002).

2.5. Knowledge gaps in breeding for iron- and beta-carotene-dense rice

2.5.1. The genotype factors that optimally determine bioavailability

Determining the bioavailability to humans of micronutrients in plant foods depends on a myriad of factors, which interact to determine the ultimate bioavailability of a particular micronutrient to an individual, eating a mixed diet, within a given environment (Fairweather-Tait & Hurrell, 1996; Welch, 2002). These factors include the combination of nutrients, antinutrients, and absorption enhancers present in the nutrient enhanced rice and the meal in which it is eaten. Because of this complexity, the data obtained using in vitro systems, animal models and test meals are always ambiguous (House, 1999; Van Campen & Glahn, 1999). Only data obtained on reducing the prevalence of micronutrient deficiencies, using feeding trials in test populations under free living conditions, can delineate the actual efficacy of using micronutrient-enriched varieties of plant foods as an intervention tool. However, it is impractical to test, in this way, the bioavailability of selected micronutrients, in the numerous genotypes of staple plant foods that can be generated in plant breeding programs (Graham et al., 2001; Graham & Welch, 1996). The ultimate proof of the biofortification strategy will lie in effectiveness studies undertaken in areas where nutritionally improved varieties will be introduced.

2.5.2. Consumer acceptance

There are various potential barriers to the adoption, for example, of Golden Rice.

- It is yellow in colour, whereas Asian consumers who stand to benefit the most from its introduction are used to eating white rice.
- There may be consumer acceptance issues, potentially related to safety, ethics and localisation of the decision-making process in agricultural development and associated processes.
- There may be concerns that technological solutions used in isolation will exclude alternative approaches, including for example, failure to harness technology to social policy.

Whether public health education initiatives can address all of these concerns is open to question. Examples of successes in programs encouraging consumers to switch from white to yellow or orange varieties of staple food crops provide some encouragement (Hagenimana & Low, 2000).

2.5.3. Relative efficiency of transgenic and conventional breeding methodologies

Much experience has been accumulated with conventional breeding and it continues to become more efficient, in part owing to new techniques developed through the use of biotechnology. Yet, clearly the capability to move genes across species holds great potential, as demonstrated by the examples presented above for rice, to accomplish goals that cannot be attained with conventional breeding. As experience is gained with biotechnology, development of transgenic varieties will become more efficient and cost-effective. It is difficult if not impossible to determine how investments should best be allocated between conventional breeding, which will give benefits now, and transgenic methods, which will generate future benefits.

2.5.4. Food and nutrient intakes stratified by country, urban-rural areas, and socioeconomic class

A rigorous ex ante evaluation of the benefits of biofortification requires the following:

- data on micronutrient status for various categories of individuals (e.g. by age, gender, income class, geographical location), matched with
- detailed knowledge of their food intakes and
- a reliably estimated empirical relationship, which ‘explains’ observed micronutrient status as a function of accurately measured levels of nutrients and other compounds in the diets.

A first attempt at such a study is described above (Section 2.3.2); however mismeasurement of food intakes and nutrient content probably leads to an underestimation of the benefit of dietary quality on micronutrient status.

2.5.5. Unintended effects

When several nutrients are lacking in diets and a single nutrient is added in significant quantities, it is unclear what the nutritional outcome will be, in the light of important interactions among nutrients, which are not fully understood. It will be important to check that there are no negative, unintended consequences of adding one or a few nutrients to the diet or of altering the levels of other compounds in foods.

2.6. Other potential contributions of GM crops

Sections 2.2–2.5 have sought to place the effort to breed for transgenic iron- and beta-carotene-dense rice
Table 2.1. Genetic modification intended to enhance the nutritive value of food crops

<table>
<thead>
<tr>
<th>Nutritive component</th>
<th>Target crop</th>
<th>Targeted gene product</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fats and oils</td>
<td>Soybean</td>
<td>Omega-3-fatty acid</td>
<td>(Kinney, 1996)</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>Stearidonic acid (SDA)</td>
<td>(Voelker, 1997; Ivy, Beremond, &amp; Thomas, 1998; Jalani, Cheah, Rajanaidu, &amp; Darus, 1997)</td>
</tr>
<tr>
<td></td>
<td>Sunflower</td>
<td>Docosahexaenoic</td>
<td>(Knutzon, 2000)</td>
</tr>
<tr>
<td>Protein</td>
<td>Rice</td>
<td>Beta-phaseolin</td>
<td>(Zheng, Sumi, Tanaka, &amp; Murai, 1995)</td>
</tr>
<tr>
<td></td>
<td>Soybean</td>
<td>Methionine enriched</td>
<td>(Kim, Kamiya, Sato, Utsumi, &amp; Kito, 1990)</td>
</tr>
<tr>
<td></td>
<td>Sweet potato</td>
<td>Essential amino acid</td>
<td>(Katsube et al., 1999)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Potato</td>
<td>Amylose and amyllopectin structure/ratio</td>
<td>(Visser et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>Cassava</td>
<td>Amylose and amyllopectin structure/ratio</td>
<td>(Visser et al., 1997)</td>
</tr>
<tr>
<td></td>
<td>Banana</td>
<td>Amylose and amyllopectin structure/ratio</td>
<td>(Schwall et al., 2000)</td>
</tr>
<tr>
<td>Carotenoids and vitamin E</td>
<td>Fruits and vegetables</td>
<td>Beta-carotene</td>
<td>(Hauptman, Eschenfeldt, English, &amp; Brinkhaus, 1997)</td>
</tr>
<tr>
<td></td>
<td>Soybean</td>
<td>Alpha-tocopherol</td>
<td>(Romer et al., 2000)</td>
</tr>
</tbody>
</table>

Table 2.2. Genetic modification intended to enhance the quality of food crops and foods

<table>
<thead>
<tr>
<th>Improved quality</th>
<th>Target crop</th>
<th>Targeted gene product</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delayed ripening</td>
<td>Fruits</td>
<td>Reduced ethylene production</td>
<td>(Theolgis &amp; Sato, 2000)</td>
</tr>
<tr>
<td></td>
<td>Vegetables</td>
<td>Maintain cytokinin levels</td>
<td>(Gan &amp; Amasino, 1995; Amasino &amp; Gan, 1996)</td>
</tr>
<tr>
<td>Enhanced appearance</td>
<td>Fruits and vegetables</td>
<td>Inhibit polyphenol oxidase; inhibit browning</td>
<td>(Bachem et al., 1994; Murata et al., 2000; Coetzer, Corsini, Love, Pavek, &amp; Turner, 2001)</td>
</tr>
<tr>
<td></td>
<td>Potatoes</td>
<td>Inhibit UDP-glucose pyrophosphorylase; inhibit starch breakdown</td>
<td>(Secor, Borovkov, McClean, &amp; Sowokinos, 1997)</td>
</tr>
<tr>
<td>Extended functionality</td>
<td>Wheat</td>
<td>Protein modification of high molecular weight glutenins; flour functionality</td>
<td>(Shewry, Tatham, Barro, Barcelo, &amp; Lazzetti, 1995; Blechl &amp; Anderson, 1996; Barro et al., 1997; Shewry &amp; Tatham, 1997; Vasil &amp; Anderson, 1997)</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>Starch modification (amylose/amylopectin ratios)</td>
<td>(Visser et al., 1991; Visser, Suurs, Steeneken, &amp; Jacobson, 1997; Heyer, Lloyd &amp; Kossmann, 1999)</td>
</tr>
<tr>
<td></td>
<td>Maize</td>
<td>Arrowroot</td>
<td></td>
</tr>
<tr>
<td>Enhanced sweetness</td>
<td>Tomatoes</td>
<td>Monellin</td>
<td>(Pennarubia, Kim, Giovannoni, Kim, &amp; Fischer, 1992)</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cucumber</td>
<td>Thaumatin</td>
<td>(Scwacka et al., 1999)</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>Canola</td>
<td>Increased stearic acid</td>
<td>(Facciotti, Bertain, &amp; Yuan, 1999)</td>
</tr>
<tr>
<td></td>
<td>Cottonseed</td>
<td>Increased stearic acid</td>
<td>(Lui, Singh, &amp; Green, 2000)</td>
</tr>
<tr>
<td></td>
<td>Soybean</td>
<td>Increased oleic acid</td>
<td>(Lui &amp; Brown, 1996)</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>Increased oleic acid</td>
<td>(Mazur, Kребbers, &amp; Tinge, 1999)</td>
</tr>
</tbody>
</table>
in the context of the need for such interventions in developing countries, the likelihood of success, alternative interventions, and costs and benefits.

Various attempts to enhance the nutritional or potential health benefits of crops or food ingredients, in other ways, have also been reported. A detailed analysis of other biotechnology-related research to improve the nutritional and health enhancing effects of food of relevance to developed as well as developing countries is not given herein, although an overview of several of these activities is presented below. The requirements for the development and assessment of such GMFs will be similar to those discussed above for GM rice. Possible genetic modification interventions of relevance to nutritional and quality factors are outlined in Tables 2.1 and 2.2.

2.6.1. Enhancing nutritional value

Strategies similar to those described above for Golden Rice have been employed to develop other crops with enhanced macro- or micronutrient content. Some of these modified crops are listed in Table 2.1; however, none of them has reached the stage of commercialisation, so it is uncertain what drawbacks or risks might be associated with each.

2.6.2. Non-nutritive active components

There is considerable private sector and consumer interest in ‘functional foods’, that is foods with special health benefits not associated with nutrients. This section gives a brief insight into how biotechnology may be used to produce healthier foods. It should be emphasised, however, that these new varieties are the subject of research that is in its earliest stages. Much fundamental research needs to be done to substantiate the proposed health benefits and associated risks, prior to the development of products with enhanced phytochemical content.

It is now widely recognised that plants contain many physiologically active components that have no classically defined nutritional role, but may have positive or adverse effects on human health. Much as is the case with carotenoids and antioxidants, consumers seem ready to seek out and use new foods for which some health claim or expectation has been described. Many of these are viewed as a ‘natural’ means to ensure good health. For the present, there are only a few food components that sound evidence links positively to health outcomes, as is the case, for example, for some phytosterols (discussed below). Nonetheless, the pathways by which some of these compounds are synthesised are being elucidated so that ultimately their levels can be manipulated. This has given rise to the new field called ‘nutritional genomics’ or ‘metabolic engineering’ (Del-laPenna, 1999, 2001).

Plant phytosterols, such as beta-sitosterol, were once used as cholesterol-lowering drugs. Recently, functional food products, such as margarines, containing phytosterols or phytostanols have appeared on the market in Europe and the USA to help lower elevated cholesterol levels. The sources of these compounds include pine tree resin and soybeans. The content of phytosterols in oilseeds has been enhanced by genetic modification (Venkatramesh, 2000). Such an improvement could increase the availability of this new functional component so it could be included in more foods.

It is easy to understand the consumer appeal of functional foods that are claimed to prevent cancer. For example, glucoraphanin, a glucosinolate found in *Brassica* vegetables, is hypothesised to have cancer-preventing effects in humans. It has been shown to induce detoxification enzymes in the liver although it is unclear if that is the mechanism by which it could prevent cancer. Glucoraphanin is not present in foods in high concentrations and it is found in foods, such as broccoli, that are not particularly popular with some consumers. Research is now being directed at increasing levels of glucoraphanin in certain vegetables. What effect this will have on the overall safety, flavour or acceptability of food will be the subject of much investigation if such plants are developed.

Flavonoids are a class of compounds, including isoflavones, anthocyanins, rutin, quercetin, and kaempferol, that are synthesized from phenylalanine via chalcone synthase and chalcone isomerase. They are present in fruits, vegetables, nuts and seeds. Flavonoids have strong antioxidant activity; both short- and long-term effects in humans of elevated consumption have yet to be demonstrated. It is hypothesised, but has not yet been proved, that the health benefits associated with diets rich in fruits and vegetables may derive, in part at least, from intake of components such as flavonoids. It has been difficult to convince consumers in some cultures, particularly children, to eat recommended quantities of fruits and vegetables. An alternative may be to help increase the intake of components such as flavonoids by enhancing their levels in fruits and vegetables, through biotechnology. Recently, the gene for soybean isoflavone synthase has been expressed in the laboratory model plant, *Arabidopsis thaliana*; the transformant non-leguminous plant produced the legume-derived isoflavone, genistin (Jung et al., 2000). Also the gene encoding chalcone isomerase has been isolated from petunia and inserted into tomato (Muir et al., 2001).

Paste processed from the transformed tomatoes contained up to 1.9 mg flavonols per gram dry weight, a 21-fold increase compared with wild controls. Furthermore, there was no negative effect on the plants and no undesirable flavours developed in the paste product.

It is widely assumed that consumers will readily accept functional foods, as people will perceive there is a benefit to health associated with such products. However, some psychological factors are important determi-
nants of food choices, and there are inter-individual and cross-cultural differences in attitudes. For example, consumer research has demonstrated that, very generally, older Americans are more positive towards ‘functional foods’ than are younger Europeans. Consumers are more tolerant of the enrichment or fortification of foods with substances that they already contain than enrichment of foods with novel substances. Individual differences in barriers to dietary change have also been identified. These include: ‘food neophobia’; the extent to which people are fatalistic about developing disease and do not adopt dietary strategies to prevent illness; and cultural factors that determine food choice. Other barriers to dietary change include factors like ‘optimistic biases’, in which people rate their own risk of developing illnesses as less than people with whom they compare themselves. Such psychological barriers have been shown to influence the extent to which individuals adopt healthy behaviours (such as healthy food choices), in line with recommendations (Frewer, Scholderer, & Lambert, in press).

In order to succeed, the development and use of functional foods should be based on documented efficacy and should take account of diverse needs and requirements of different populations and demographic groups, as well as cultural factors that determine food choices. In broad terms, simply providing information about health benefits is unlikely to be an influential strategy for changing well-established consumer behaviours.

2.6.3. Improving quality traits

Biotechnology offers the possibility to produce grains, fruits and vegetables that retain their fresh appearance longer and have a longer useful shelf life than non-modified products. Other targets for modification include alterations in flavour, taste, texture and viscosity. Changes can be directed at alteration of the expression of a key enzyme or introduction of a new protein or enzymatic activity. For example, antisense RNA has frequently been employed to block the synthesis of key enzymes. Some examples, representative of the scope of the modifications that have been reported, are presented in Table 2.2.

For example, fresh fruits and vegetables are relatively perishable commodities that have limited shelf lives. Moreover, fresh produce and fruits must often be picked in a green or unripened state in order to allow time for shipping, marketing and, ultimately, ripening prior to sale. Plant breeders have sought for many years, with only limited success, to develop varieties that have delayed ripening and extended shelf lives. The introduction of molecular breeding techniques has allowed the targeting and modification of pathways involved in ripening and senescence in certain crops. In fact, the very first product of biotechnology to be approved as a food was designed to enhance freshness. Softening of tomatoes can be delayed by insertion into tomatoes of an antisense mRNA that inhibits synthesis of an enzyme, polygalacturonase, that breaks down pectin. The resulting ‘Flavr Savr™’ tomato was sold as whole fresh fruit in supermarkets while a similar tomato was used in processed tomato products in the UK (Roller & Harlander, 1998). The products have been withdrawn from the market because they were unprofitable.

2.6.4. Removing allergens and antinutrients

The primary food allergens in a plant are frequently major proteins. One approach to reducing allergenicity is to knock out the biosynthesis of the offending protein. A second approach is to modify the structure of the offending protein so that it no longer contains the epitopes responsible for the undesirable immune responses that result in allergic signs and symptoms. The first approach has been applied to rice. The content of the major allergenic protein in rice has been successfully reduced; however, it is not known if this results in decreased allergenicity in humans (Tada et al., 1996). Peanut allergen (Bannon, Shin, Maleki, Kopper, & Burks, 1999) and potato allergen (Alibhai et al., 2000; Astwood, Alibhai, Lee, Fuchs, & Sampson, 2000) have been modified by protein engineering to remove epitopes associated with allergenicity. A more general method of disarming potential food allergens is also being tested (Buchanan et al., 1997). Thioredoxin is a regulatory disulphide protein that is widely distributed in nature. It has been found to reduce disulphide bonds in allergens, and the reduced allergens are less allergenic. Research is underway to determine if insertion of a gene for thioredoxin biosynthesis into wheat will reduce its allergenicity without interfering with the functionality of the wheat proteins.

Members of the Brassica family, such as cabbage, Brussels sprouts and broccoli, rapeseed and mustard contain a family of compounds called glucosinolates. Glucosinolates can be hydrolysed to bitter tasting and potentially toxic isothiocyanates. Extensive plant breeding was required to produce varieties of rapeseed and mustard with greatly reduced levels of glucosinolates. The meal remaining after oil extraction from these varieties can be used in animal feed. Glucosinolates are part of the plant’s defence system against insect pests and pathogens. Attempts are now being made to develop plants that have high glucosinolate levels in leaves and seedpods but much reduced levels in the oilseeds (Vageeshbabu & Chopra, 1997). Solanine and chaconine are naturally occurring glycoalkaloid toxins that are found in potatoes and other solanaceous plants. While toxic to humans, the com-
pounds are destroyed by cooking. It has been reported that insertion of an invertase gene from yeast into potato reduces the glycoalkaloid content (Engel, Blaas, Gabriel, & Beckman, 1996). The exact mechanism for this inhibition is not known and further work seeks to establish if the effect is caused by metabolic interference with the sugar moieties needed for biosynthesis of glycoalkalooids. It is also possible to use antisense RNA to block synthesis of a gene that codes for a key enzyme in glycoalkaloid synthesis.

Fumonisin is a mycotoxin that is produced by the fungus *Fusarium*. It accumulates in maize that has become infected at the site of damage by insects such as the European corn borer. Although the fumonisin content of maize is approximately proportional to the level of insect damage, the temperature and humidity of storage can affect the level of mycotoxin accumulation. Levels of fumonisin in grain should be carefully controlled since fumonisin B1 is a possible human carcinogen (IARC, 2002). It has been linked to oesophageal cancer in humans who consume high levels of fumonisin-contaminated maize. Fumonisin causes liver cancer in rats (Gelderblom, Abel, et al., 2001) and liver and kidney damage in non-human primates (Gelderblom, Seier, et al., 2001); it is also known to be acutely toxic to livestock. De-esterase genes have been transferred from yeast into maize for the purpose of detoxifying fumonisin (Duvick, 2001).

As *Bt*-maize is resistant to attack by insect pests, levels of fumonisin in *Bt*-maize are consistently greatly reduced over levels observed in its conventional counterpart (Cahagnier & Melcion, 2000; Dowd, 2000; Munkvold, Hellmich, & Rice 1999). In a 3-year study conducted in northern Italy, where fumonisin contamination of corn is a major problem, fumonisin levels in *Bt* corn were greatly reduced compared with traditional hybrids (Pietri & Piva, 2000). Reductions in the fumonisin content of maize represent a significant enhancement in food and feed safety and will also reduce post-harvest losses associated with the destruction of contaminated grain.

### 2.7. Standards for food use

Many of the potential GM products that are described herein have altered macro- or micronutrient composition. Modifications are intended to enhance nutritional value or alter the quality characteristics of the food. These new plant varieties contain altered contents of macronutrients, micronutrients or non-nutritive compounds of special interest that are ordinarily consumed in the human diet.

These new crops are intended for distinct uses. Products such as Golden Rice are intended to replace or partially replace a commodity crop, in regions where populations consuming rice are at risk of vitamin A deficiency. Many of the plant developments described in this chapter will be segregated during cultivation and offered for sale to consumers, based on their enhanced nutritional or quality characteristics.

Any type of crop whose nutritional composition has been altered, whether by traditional or biotechnological means, must be shown to be stably maintained; furthermore the crop should not interfere with or negatively impact on other cropping systems or the environment. It will also be important to monitor the nutritional status of populations that have adopted new nutritionally enhanced crops.

Also, any type of crop whose nutritional composition has been altered, whether by traditional or biotechnological means, should pose no unique environmental or food risk. The most significant challenge will be to predict changes in the dietary intake of specific nutrients when these products are introduced into the marketplace. It may be inadvisable to market products that contain elevated levels of nutrients that could be toxic to the 99th percentile of consumers. National food consumption surveys may be useful in monitoring changes in dietary intake.

### 2.8. Conclusions

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

- To be of value plant breeding has to be demonstrated to be safe (see below) and more cost-effective than alternative interventions already in place to reduce micronutrient undernutrition. Such cost-effectiveness is anticipated, in large measure because of the far-reaching impacts of plant breeding. A relatively small, fixed, initial investment in research could benefit the health of millions of poor people in developing countries all over the world, and at the same time could improve agricultural productivity on lands that are presently among the least productive. Nutrient-dense varieties of crops can be developed for a fraction of the recurrent estimated annual costs of supplementation programmes in developing countries (for example US $1.65 billion for vitamin A supplementation programmes) and can reach far more people, as the benefits can be extended across countries and time.

- There should 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, biotechnology may also be a more efficient way to increase trace mineral density.
Where biotechnology is superior to conventional plant breeding, it must be recognised that the same conditions as that apply to products developed via conventional plant breeding, must also be met, as follows:

- there should be no serious, negative agronomic consequences associated with the characteristic being added;
- any noticeable changes in the colour, taste, texture, cooking qualities, and other features associated with the characteristic being added should be acceptable to the consumer;
- the characteristic being added should result in a measurable improvement in the nutritional status of the malnourished, target population; and
- there should be no unacceptable nutritional effects.

The conditions, relating to safety, acceptability and efficacy, in particular, have yet to be firmly established. Nutritionally improved transgenic foods have not been fully developed and tested for their potential to improve micronutrient status, even using in vitro techniques and animal models, let alone in human subjects with micronutrient deficiencies.

Definitive proof of the benefit of nutritionally improved, transgenic foods obviously depends on further development, release and eventual impact evaluation, all of which are many years into the future. Nevertheless, in the shorter term, lessons learned through initial experiences with conventional breeding efforts show just how cost-effective a biofortification strategy can be. Clearly, the nutritional quality of staple foods can be substantially improved using transgenic methods compared with what can be accomplished using conventional breeding. Knowledge in the area of nutritional genomics should expand rapidly in the future and more efficient techniques for producing transgenic plants will be developed. There are potentially substantial benefits to the poor in developing countries in relation to costs.

Characteristics of food, such as appearance, taste, and colour, which are important to consumers in both developed and developing countries, can also be improved through biotechnology; however, such research currently probably benefits wealthy consumers in developed countries 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. Thus, the best and final solution to eliminating undernutrition as a public health problem 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 to be realised, informed government policies, and a relatively large investment in agricultural research and other public and on-farm infrastructure.

In conceptualising solutions for a range of nutritional deficiencies, interdisciplinary communication between plant scientists and human nutrition scientists holds great potential. Human nutritionists need to be informed, for example, about the extent to which the vitamin and mineral density of specific foods, as well as compounds that promote and inhibit their bioavailability, can be modified through plant breeding. Plant breeders need to be aware of both the major influence that agricultural research may have had on nutrient utilisation in the past (e.g. the bioavailability of trace minerals in modern varieties versus bioavailability in traditional varieties), and the potential of plant breeding for future improvements in nutrition and health.

2.9. References


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