Prospects for the biofortification of grains by genetic engineering or plant breeding

Richard F. Hurrell, Swiss Federal Institute of Technology Zurich, Institute of Food Science and Nutrition, Laboratory of Human Nutrition, P.O. Box 474, CH-8803 Rüschlikon, Switzerland, Phone + + 41-1-704 57 01, Fax + + 41-1-704 57 10, Email richard.hurrell@ilw.agrl.ethz.ch

Many populations survive largely on plant-based diets with little or no meat or dairy products. Monotonous consumption of cereal staples, roots or pulses, in the absence of animal tissue can lead to deficiencies in essential vitamins and minerals. Despite improvements over the past 50 years, over 2 billion people, one-third of the world's population suffer from iron, zinc and/or vitamin A deficiencies (1). Most of these people are infants, children and women living in the poorer developing countries of Asia and Africa. Iron deficiency is the most widespread nutrient deficiency and can decrease mental and psychomotor development in children (2), increase both morbidity and mortality of mother and child at childbirth, decrease work performance, and decrease resistance to infection (3,4). Zinc deficiency reduces growth, sexual maturity and immune defense (5) and is particularly important in children in the developing countries where it can increase incidence of diarrhea and respiratory infections (6). Vitamin A deficiency affects especially pre-school children. Many go blind and others die from minor diseases such as measles because of a reduced immune system (7).

Strategies to combat micronutrient deficiencies

The three most widely recognized strategies for reducing micronutrient malnutrition are supplementation with pharmacological preparations, food fortification and dietary diversification (8). Supplementation is relatively expensive and often has poor compliance, particularly with iron, because of adverse side-effects. It is the most common approach to prevent vitamin A deficiency. Food fortification is usually considered the best long term strategy for the prevention of micronutrient deficiencies, however iron fortification of cereals has been largely unsuccessful. This is because the most bioavailable iron compounds often lead to unwanted sensory changes and those compounds which are organoleptically acceptable are poorly absorbed (9). In addition, fortified foods rarely reach remote villages. Food diversification may also be difficult in developing countries for economic and social reasons. A new strategy for combating micronutrient malnutrition is the biofortification of food staples such as cereal grains by plant breading or genetic engineering (10,11).

Micronutrient dense grains can be produced by classical plant breeding techniques or by using molecular methods to transfer genes across species. Biofortification of grains is a mass fortification strategy that reaches all population groups including those in remote rural villages. It is sustainable as it does not depend on political will, it makes use of abundant, untapped soil mineral resources and, once developed, has no running or monitoring costs (12). The benefit to cost ratio has been calculated as 19 for the development of an iron-rich rice as a result of better iron nutrition, which is a similar ratio to that found for iron fortification (12).

Genetic engineering

Although much of the research in plant biotechnology in recent years has concentrated on increasing yield by improving resistance to environmental stresses, pests and pathogens (13), its recent application to improve the nutritional content of staple food crops has perhaps the greatest potential benefit to global health. The major technical breakthrough came with the use of genetic engineering techniques to produce rice grains containing β-carotene, the major precursor of vitamin A (14). Ye et al. (14) inserted the β-carotene synthetic pathway into rice endosperm via the Agrobacterium mediated introduction of the genes for 3 enzymes; phytoene synthase and lycopene β-cyclase (from daffodils), both under the control of the endosperm specific glutelin promoter, together with a bacterial phytoene desaturase The 'golden' rice grains contained 1.6-2.0 μg/g β-carotene, which is still rather modest in nutritional terms. Assuming that 6 μg β-carotene is converted into 1 μg retinol equivalents (REs) (15), 1.2 kg of 'golden' rice per day would be necessary to provide all the recommended vitamin A intake of a 4-8 year old child (400 μg RE/d). The key question, however, is the bioavailability of the newly-introduced β-carotene which has still to be measured. While the bioconversions of β-carotene to vitamin A is 6:1 in fruits, it is 24:1 in vegetables such as spinach due to poor release during digestion (16). The conversion factor is not known for β-carotene introduced into 'golden' rice.

There are several genetic engineering approaches that could be used to increase iron and zinc contents in grains. These include increasing uptake from the soil by introduction of genes coding for siderophores, genes for the production of chelating agents and reducing agents, enzymes, root transporter proteins, or status signaling proteins; improving transport in xylem, and improving phloem transport from the leaf to the seed; and finally increasing storage (17). Increasing the concentration of the storage proteins phytoferritin and metallothionein could increase the concentration of iron and zinc respectively. Samuelsen et al. (18) recently reported a transgenic tobacco plant expressing a yeast ferric reductase in the roots, which increased leaf iron content by 50%, and Goto et al. (19) reported a 2-3 fold increased in iron in transgenic rice grains expressing the soybean ferritin gene.

A strategy to improve iron and zinc nutrition that is focussing only on increasing the Fe and Zn content of grains, however, by itself may not be enough. This is because other food components have a major impact on Fe and Zn absorption. Phytic acid, omnipresent at about 1% in grains and legume seeds, is a potent inhibitor of iron and zinc absorption. In addition, Fe absorption is decreased by phenolic compounds and increased by ascorbic acid and muscle tissue (20). The influence of muscle tissue is thought to be due to the cysteine-containing peptides formed during digestion.

Lucca et al. (21) have reported on a combination of three genetic engineering approaches to improve the bioavailability and level of iron in rice grains. Firstly a reportedly thermostable phytase (22) from Aspergillus fumigatus, active at gastrointestinal pH, was introduced using an Agrobacterium mediated transformation. In addition, a ferritin gene from Phasolus vulgaris was introduced and the endogenous cysteine-rich metallothionen-like protein in rice was overexpressed. All three genes were expressed in the endosperm. The phytase activity increased 7 fold and phytic acid in uncooked rice was completely degraded during simulated intestinal digestion. Unfortunately, the heat stability of the phytase was lost after expression and the phytase was destroyed on cooking the rice. Iron content, however, was doubled and the cysteine content increased 7 fold. The influence of these changes on iron bioavailability in man, however, remains to be investigated.

Plant breeding

For staple crops, thousands of different genotypes of rice, wheat and maize exist, and genotype influences the nutrient content of plants and seeds (23). In a recent screening of germ plasm, a high variation in iron and zinc content was reported in wheat (25-56mg Fe and 25-65mg Zn/kg), rice (7-23mg Fe and 17-52mg Zn/kg) and maize (13-160mg Fe and 11-95mg Zn/kg) (17). Moreover the amount of iron and zinc appear to be highly correlated with each other (24). A major difficulty remains, however, in that the speciation of this additional mineral content is not known, nor is it known where the minerals are present in the grain. Additional, minerals are preferred in the endosperm, as milling or polishing will remove minerals in the outer layers of the seed. Bioavailability may also be an issue if the minerals are associated with phytate or, in the case of iron, if it is contained within phytoferritin. The bioavailability in man of phytoferritin Fe is not known although bovine ferritin iron is about 40% as well absorbed as iron salts. (25) It has recently been reported that experimentally mineral enriched wheat increased yield on mineral-poor soil in Bangladesh due to increasing vigor and viability (26), however, in maize, Fe and Zn have been reported to correlate negatively with grain yield (27).

A decrease in the level of phytic acid has been proposed as a plant breeding strategy to improve mineral bioavailability. Phytic acid is a source of phosphorus, minerals and energy for the seedling. There is concern that lowering phytate might reduce seedling vigor in low fertile soils (17). Chemically induced mutants in maize, barley and rice have been generated (28). These single gene, low phytic acid mutations cause the seed to store most of the phosphorus as inorganic phosphorus instead of phytate phosphorus. A maize mutant with a ca. 65% reduction in phytate was backcrossed into elite maize inbred lines without any marked alterations in important agronomic factors. The improvement in mineral absorption, however, is at best modest. Phytic acid is a potent inhibitor of iron absorption and in soy and wheat must be reduced by > 90% to get a 3-4 fold increase in iron absorption in man (29,30). The mutant maize increased iron absorption 50% in one study (31) and not at all in a second study (32). The reported improvement in zinc absorption from a polenta meal from 17% to 30% would, however, be regarded as being much more useful (28).

Evaluation of potential breeding strategies

Biofortification is an exciting new strategy to increase the micronutrient levels in grains. After a series of pioneering studies, there is now an immediate need to make human bioavailability studies with grains enriched in β-carotene, iron and zinc, and especially a need to make efficacy studies to demonstrate that the modified grains improve the micronutrient status in human populations. At the same time, speciation studies are needed to identify in what form and where iron and zinc are situated in the grain. This is particularly important in relation to bioavailability and losses during milling.

Phytic acid is the major obstacle to iron and zinc bioavailability from grains and it is doubtful whether a decrease in the level of phytate in the grain, while maintaining seedling vigor, will have a major impact on iron nutrition. The introduction of phytases is more promising, but technically it is still not possible to express a heat-stable phytase which would resist cooking and degrade phytic acid during digestion. In relation to improving iron nutrition, the introduction of ascorbic acid would be the preferred strategy but is also technically still not possible. The introduction of cysteine-containing metallothionein, as a replacement for muscle proteins, is an interesting approach but is not guaranteed to improve iron absorption and must be tested. The introduction of phytoferritin is the most plausible strategy at the present time, however, iron absorption from phytoferritin has never been measured in man.

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