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Biofortified crops to alleviate micronutrient malnutrition

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Micronutrient malnutrition affects more than half of the world population, particularly in developing countries. Concerted international and national fortification and supplementation efforts to curb the scourge of micronutrient malnutrition are showing a positive impact, alas without reaching the goals set by international organizations. Biofortification, the delivery of micronutrients via micronutrient-dense crops, offers a cost-effective and sustainable approach, complementing these efforts by reaching rural populations. Bioavailable micronutrients in the edible parts of staple crops at concentrations high enough to impact on human health can be obtained through breeding, provided that sufficient genetic variation for a given trait exists, or through transgenic approaches. Research and breeding programs are underway to enrich the major food staples in developing countries with the most important micronutrients: iron, provitamin A, zinc and folate.

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Introduction

The adage 'health comes from the farm, not the pharmacy' is at the heart of ongoing international biofortification research and breeding programs.[3] Biofortified crops are being developed with increased bioavailable concentrations of essential micronutrients to be deployed to consumers through the traditional conduits used by agriculture and food trade. This contrasts with industrial fortification (e.g. iodine in salt or vitamin A and D in margarine) and supplementation (e.g. vitamin A capsules) programs, which rely on industrial processing, and specialized distribution channels or access to health systems and markets.

Biofortification relies on the plant's biosynthetic (vitamins) or physiological (minerals) capacity to produce or accumulate the desired nutrients. Biofortified crops can be obtained through breeding, provided sufficient genetic variation is present in the diversity spectrum or by exploiting transgressive segregation or heterosis. In the absence of such variability then genetic modification offers a valid alternative.

This paper will discuss biofortification in the context of micronutrient malnutrition (MNM), a major health burden affecting more than half of the world's population, especially in developing countries. For functional foods designed primarily for people in developed market economies to live healthier lives or deal with modern, nutrition-related civilization diseases, such as obesity, we refer the reader to recent and comprehensive reviews [1–3].

Micronutrient malnutrition today

The global significance of MNM – also known as hidden hunger – came to the attention of the nutrition community as recently as the mid 1980s, when protein-energy malnutrition was widely seen as the culprit of the world's nutrition problems [4]. The 1990 World Summit for Children, convened by governments and facilitated by the UN with support from UNICEF, the World Bank, WHO, FAO, UNDP, CIDA and USAID, was a landmark event in the fight against MNM. Three of the summit's goals directly addressed the elimination or significant reduction of deficiencies in iron, vitamin A, and iodine by the year 2000, thus providing development agencies with the necessary political mandate. Activities towards achieving these goals focused on traditional public health intervention strategies, and resulted in measurable impacts; however, the year 2000 targets are still far from being met [5•,6•,7–9].

In 2001, the General Assembly of the UN adopted the Millennium Development Goals (MDGs) resolution which aims to eradicate or alleviate the world's greatest health and poverty issues by 2015 [10]. Fighting MNM is an integral component of three of the eight MDGs: (1) eradication of extreme poverty and hunger; (4) reduction of child mortality; and (5) improvement of maternal health [11•]. Micronutrient supply is among the main preventive and curative interventions with proven and substantial capability to contribute towards achieving these goals [12]. Widespread MNM also results in an

[3] HarvestPlus (http://www.harvestplus.org), and Challenge #9 under the Grand Challenges in Global Health initiative of the Bill & Melinda Gates Foundation (http://www.gcgh.org).

enormous negative socio-economic impact at the individual, community, and national levels [7].

Even though insufficient intake of any essential micronutrient will result in metabolic impairment of individuals, potentially increasing morbidity and death rates, numerous national and regional surveys have identified iron, vitamin A and iodine as most vital for global human health, among almost 30 essential micronutrients [13]. Micronutrients such as zinc and folate, are acquiring similar status as statistics accumulate (see Box 1).

The case for biofortification

Traditional public health interventions, like supplementation and industrial fortification, have notably reduced MNM-induced morbidity and mortality worldwide. Nonetheless, attainment of the MDGs is not on track, mainly because classical interventions require infrastructure, purchasing power, or access to markets and health-care systems for their success, often not available to people living in remote rural areas. Food fortification programs rely on widely distributed, industrially processed food items, usually unaffordable for half of the world's poor living on less than $ 2 per day, and much less another 30% who live on less than $ 1 per day!

Experience with vitamin A supplementation programs revealed that coverage achieved over the last decade in 103 priority countries has stagnated at 58%, with high year-to-year fluctuation [6]. In India, the 'Nutritional Anaemia Control Programme' has been in place since 1970 with little impact, because of mismanagement, underfunding, logistic problems, and poor compliance [14]. In recent years, coverage with iron-folate supplements was around 30% for pregnant women and 10% for adolescent girls [15].

While undoubtedly money spent on these interventions is money well spent, both intervention strategies depend on uninterrupted funding. But the flow of funds can falter, as exemplified by the case of Burmese refugees along the Thailand–Burma border, where a recent interruption of

Box 1 Micronutrient malnutrition

In 2000, the World Health Report identified iron, vitamin A, zinc and iodine deficiencies as the most serious health constraints worldwide [45]. Even though worldwide prevalence of folate deficiency is not well documented, it is included here, because of its central role in child development and its amenability to biofortification. Additional micronutrients being considered for intervention purposes but not discussed here are vitamins C, D, and various B vitamins, as well as the minerals selenium, calcium, and fluoride. The genetic potential for increasing the concentrations of bioavailable Fe, Zn, provitamin A carotenoids, selenium, and iodine in edible portions of several major staple food crops has been reviewed recently [43,46•].

Iron is a redox-active constituent of the catalytic site of heme and non-heme iron proteins. More than one-third of the world's population suffer from anaemia; half of it caused by iron deficiency [17]. Endemic infectious diseases exacerbate the incidence of iron deficiency anaemia in developing countries. Iron deficiency adversely affects cognitive development, resistance to infection, work capacity, productivity, and pregnancy. Children of anaemic mothers have low iron reserves, requiring more iron than is supplied by breast milk, and suffer from growth impairment [17]. It is estimated that 800,000 deaths are attributable to iron deficiency anaemia annually. Attaining the goal of reducing iron deficiency by one-third by the year 2010, compared to 2000 levels in most developing countries is unlikely [47].

Vitamin A denotes a group of C_{20} carotenoid derivatives (retinal, retinol and its esters, and retinoic acid), which play an essential role in vision, immune response, epithelial cell growth, bone growth, reproduction, maintenance of the surface linings of the eyes, embryonic development, and regulation of adult genes. An early symptom of vitamin A deficiency is night blindness. Structural alterations of the conjunctiva and the cornea (xerophthalmia, keratomalacia) may follow, and subsequent inflammation and infection results in irreversible blindness. Depression of the immune system increases the severity of measles and diarrhoea, leading to a ninefold increase in child mortality, which is apparent even before the appearance of xerophthalmia. An estimated 127 million preschool children are affected by vitamin A deficiency, with 250,000–500,000 becoming blind every year, half of which die within 12 months of losing their sight. Ninety percent of the world's annual child deaths—more than 9 million—occur in only 42 developing countries. A combination of simple and effective nutrition interventions such as breastfeeding, complementary feeding, vitamin A, and zinc supplementation could prevent about 25% of these deaths [48].

Iodine is a component of thyroid hormones. Iodine deficiency disorders are often quoted as the single greatest cause of preventable brain damage in the foetus and infants, and retarded psychomotor development in young children. Goitre and cretinism are the most visible manifestations of iodine deficiency. It is estimated that almost one billion individuals suffer from goitre, with more than half of these living in Asia. An estimated 16.5 million people worldwide suffer from cretinism, and it is likely that another 49.5 million suffer less severe, though still measurable, forms of brain damage because of iodine deficiency [49].

Zinc is involved in RNA and DNA synthesis, and is a constituent of many zinc-containing enzymes critical to cellular growth and differentiation. While mild to moderate zinc deficiency is common throughout the world [50], one third of the world's population at high risk live in low-income countries, according to the International Zinc Nutrition Consultative Group (URL http://www.izincg.org). Zinc deficiency leads to impaired growth, immune dysfunction, increased morbidity and mortality, adverse pregnancy outcomes, and abnormal neuro-behavioural development. Zinc deficiency is directly related to the severity and frequency of diarrhoeal episodes, a major cause of child death [51]. The body of evidence on zinc deficiency has accumulated to the degree that zinc fortification has been jointly recommended by WHO and FAO [5].

Folate, or vitamin B_9, is an essential coenzyme involved in one-carbon metabolism, together with vitamin B_{12}. Folate deficiency is associated with a higher risk of newborns with neural tube defects, spina bifida, and anencephaly, and an increased risk of cardiovascular diseases, cancer, and impaired cognitive function in adults. Mandatory fortification of wheat flour with folic acid in the United States in 1998 was followed by a significant reduction in the prevalence of neural tube defects [52]. Folate deficiency also causes widespread megaloblastic anaemia during pregnancy and often exacerbates already existing iron deficiency anaemia [53]. Folate deficiency causes at least 210,000 severe birth defects every year, although the global prevalence of folate deficiency is inaccurate, because of sparse data [54].

the programme led to an immediate drop in micronutrient status and consequently to health deterioration [16]. The cost per life saved is low in economic terms, when compared to WHO and World Bank benchmarks [17•,18], but these interventions perpetuate precarious dependencies. The cost per vitamin A capsule is $ 0.10, but after including logistic and distribution costs the price tag jumps to $ 1 per capsule. At present, donated capsules are distributed at a rate of 500 million per year [19].

Cost effectiveness of biofortification has been calculated for β-carotene, iron, and zinc as part of ex-ante socio-economic impact analyses for a number of target countries [19–21]. Calculations involve the use of optimistic and pessimistic technology adoption and efficacy scenarios, together with national and regional surveys of the status quo, thus providing a predictive measure of the potential benefits of an intervention on public health. In all cases, the costs of biofortification consistently constitute only a fraction of supplementation costs, and moreover, after the initial development and adoption phases, the use of biofortified crops requires little more than the costs for reliable seed production systems and deployment. The costs of breeding are moderate – in the range of $ 4 million per variety spread over 10 years – amounting to approximately 0.2% of the global vitamin A supplementation expenditures in the scenario described above over the same period [22•]. The development and regulatory approval of a transgenic crop can be 5–8 times higher [23,24], still representing only a fraction of the sustained costs necessary for a classical public health intervention.

Crop-based micronutrient delivery – chances and constraints

Plants are extremely versatile biochemical factories, capable of synthesizing a nearly full complement of essential dietary micronutrients; however, these are unevenly distributed among different plant parts. Iron content of a rice leaf, for instance, is high (100–200 ppm) but very low in the polished rice grain (3 ppm). Similarly, provitamin A carotenoids are present in rice leaves only. Unfortunately, poor people live predominantly on starchy staples such as rice, wheat, maize, or cassava, that do not provide the biochemical diversity needed for a healthy life. Food diversification is the most desirable approach for preventing MNM, but tragically poverty stands in its way, and improvement of living standards remains elusive with an ever-growing world population and geopolitical turmoil. This is where biofortification comes into the picture.

Breeding targets in biofortification are set to achieve measurable health impacts as determined by nutritionists [25]. Targets must account for local consumption levels of staples, as well as retention in typical food preparations and bioavailability of micronutrients in those crops. For example, in Africa the β-carotene levels in popular varieties of cassava, maize, and sweet potato, are close to nil. Based on available genetic variability and typical daily dietary intakes, breeders envisage a maximally attainable level of 20 ppm β-carotene for maize and cassava in the short to medium term. High β-carotene South American sweet potato germplasm is being used to improve African materials, and varieties with the full target increment are already being disseminated in Africa [26].

Based on mineral content variability within selected species, the expected increase in iron in adapted varieties in the short to medium term is about 6 ppm for milled rice, 23 ppm for wheat, and 60 ppm for *Phaseolus* beans [27]. Increases in zinc concentration may reach 22 ppm for rice, 24 ppm for wheat, and 20 ppm for beans [25].

Molecular breeding and transgenics

Many traits needed in biofortification programs can be found by exploring the genetic variation in germplasm collections. Advances in nutritional genomics [28•] and efficient molecular marker techniques allow tracking complex traits along the breeding process. Powerful new techniques, such as TILLING – saturating metabolic pathways with mutations and subsequently identifying the genes involved [29] – add to the arsenal of molecular and genomic tools, such as the increasing number of fully sequenced genomes.

Should the desired trait be unavailable within the species or when the crop is not amenable to conventional breeding approaches, as in bananas, breeders can resort to transgenes from various sources. Targets for transgenes include redistributing micronutrients between tissues, increasing the efficiency of biochemical pathways in edible tissues, or even the reconstruction of selected pathways. Some strategies, rather than increasing production or accumulation of micronutrients, might involve the inclusion of factors that increase bioavailability of micronutrients.

A case in point is *Golden Rice*, in which the carotenoid biosynthetic pathway has been reconstituted in non-carotenogenic endosperm tissue, as a means to deliver provitamin A [30–32]. Reconstitution of the pathway in other crops depends on the metabolic bottleneck, as illustrated by canola, tomato and potato [33–37]. The Grand Challenges in Global Health initiative of the Bill & Melinda Gates Foundation is funding biofortification projects on banana, cassava, and sorghum for Africa. They all include efforts to produce and accumulate carotenoids and other micronutrients in these crops. While there is genetic variation for this trait in banana and cassava, the former is not amenable to breeding because of lack of sexuality, and the latter is usually propagated vegetatively because its high heterozygosity prevents varietal recovery through backcrosses, thus making the transgenic approach attractive in both cases.

There are further encouraging examples of how transgenic approaches can complement ongoing breeding efforts and provide the urgently needed biofortified crops to feed the burgeoning world population with nutritious food. These include recent work on tomato, which boosted folate accumulation 15-fold by tweaking a highly compartmentalized pathway [38], a feat also shown to work in rice grains [39]. Also, iron content in rice grains was doubled by the overexpression of bean ferritin [40,41]. These relatively moderate gains from increased iron storage capacity most probably reflect the complex mechanisms of iron mobilization [42•,43]. A total of 43 genes belonging to five protein families are thought to be involved in rice [44].

Conclusions

Despite major concerted international efforts, eradication of MNM has remained a widespread and persistent global health problem in developing countries, where it continues to exact an enormous toll on individuals, populations, and society [7]. Food-based approaches – designed to increase micronutrient intake through the diet – represent the most desirable and sustainable method of preventing MNM. Ideally this should be achieved through food diversification, but radical improvements of the geopolitical situation in the near future cannot be expected, and practicable and cost-effective solutions to the problem are needed. Biofortification offers a long-term, sustainable, food-based solution for a world population that will reach nine billion in coming years. This poses a dual challenge to scientists developing biofortified crops, that is seeking a sustainable increase in calorie production and furnishing staple crops with the necessary micronutrients to satisfy the physiological needs of the poor. In measuring up to this challenge, biofortification also addresses socio-economic and socio-political needs, thus contributing towards equitable development.

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