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Food Biofortification—Reaping the Benefits of Science to Overcome Hidden Hunger

A paper in the series on The Need for Agricultural Innovation to Sustainably Feed the World by 2050

CHAPTER 1. JUSTIFICATION FOR BIOFORTIFICATION

Introduction

Biofortification is a process of increasing the density of minerals and vitamins in a food crop through conventional plant breeding, genetic engineering, or agronomic practices (primarily use of fertilizers and foliar sprays). Biofortified staple food crops, when substituted consistently for non-biofortified staple food crops, can generate measurable improvements in human nutrition and health.

This monograph describes the progress made in developing, testing, and disseminating biofortified staple food crops, primarily through the use of conventional plant breeding, summarizing the activities of two consortiums of inter-disciplinary collaborating institutions led the HarvestPlus program and the International Potato Center (CIP).

Two women in Zambia make nshima from vitamin A maize flour during an agricultural expo in 2015. Nshima is a thick porridge made from corn meal and water. (Photo courtesy of HarvestPlus.)

We focus on laying out the evidence base proving the effectiveness and impact to date of biofortified crops. Results of a large number of nutritional bioavailability and efficacy trials are summarized (Chapter 2), crop development techniques and activities are presented and variety releases documented for a dozen staple food crops in low and middle income countries (LMICs) in Africa, Asia, and Latin America (Chapter 3), and strategies for promoting the uptake of specific biofortified crops are discussed, concurrent with policy advocacy to encourage key institutions to mainstream the promotion, and use of biofortified crops in their core activities (Chapters 4 and 5). Statistics will be presented on numbers of farm households adopting biofortified crops (Chapters 3 and 4), now available to farmers in 40 low and middle income countries (LMICs). Each section will outline the way forward on additional future activities required to enhance the development and impact the biofortification through conventional plant breeding.

No biofortified staple food crop developed through transgenic techniques has been fully de-regulated for release to farmers in LMICs. Yet transgenic techniques hold the potential for a several-fold increase in the impact/benefits of biofortified crops. This potential is described in Chapter 6 which discusses developmental research already completed, including achieving higher densities of single nutrients than is possible with conventional breeding, combining multiple nutrient traits in single events, slowing down/reducing the level of degradation of vitamins after harvesting, and combining superior agronomic traits with nutrient traits in single events.

A final chapter summarizes and discusses key questions and issues that

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will influence the ultimate mainstreaming of biofortified crops in food systems in LMICs and will allow maximization of the benefits of biofortification.

This introductory chapter outlines the landscape for use of biofortified crops in LMICs. What are the extent and consequences of the public health problem of mineral and vitamin deficiencies? What are the underlying causes, related especially to diets and food systems? Very importantly, what is the economic justification for investing in biofortification to be included in the mix of current interventions? Institutional issues related to funding and coordination also will be discussed briefly.

The Problem: The Extent and Consequences of Mineral and Vitamin Deficiencies in LMICs

An estimated 2 billion people in the developing world suffer from the effects of micronutrient malnutrition, widely known as hidden hunger. Among the micronutrients, the deficiencies of iron, zinc, iodine, and vitamin A are most widespread and severe, while deficiencies of folates, vitamin D (Cashman 2020), thiamine (B1) (Johnson et al. 2019), and selenium (Ibrahim et al. 2019) are also growing concerns.

Preschool children, adolescent women, and in general, women of reproductive age are most vulnerable due to their higher requirements for rapid growth and reproduction, respectively. The Global Disease Burden 2015 estimates that around 1.2 billion people are affected by iron deficiency anemia (Kasenbaum 2016), which leads to impairment of cognitive function in preschool children, lowered ability to perform physical work, and higher mortality during childbirth.

At risk for zinc deficiency are 1.2 billion people, and 116,000 deaths among preschool children are attributed to zinc deficiency due to weakened immune systems (Black et al. 2013). Vitamin A is essential for cell differentiation, strong immune systems, and good vision. Deficiency results in growth faltering, higher risk of mortality, damage to mucous membranes, reproductive disorders, eye damage—and ultimately blindness. In LMICs, 30% of preschool-age children and more than 19 million pregnant women are vitamin A deficient. In 2013, 105,700 childhood deaths in LMICs were attributed to vitamin A deficiency (Black et al. 2013).

Most people suffer from multiple nutritional deficiencies, which can further aggravate the negative health consequences. Global losses in economic productivity to due macronutrient and micronutrient deficiencies account for 2% to 3% of GDP (World Bank 2006) at a global cost of $US 1.4 to 2.1 trillion USD per year (FAO 2013; von Grebmer et al. 2014).

Poor Dietary Quality, A Primary Cause of Mineral and Vitamin Deficiencies

Over the last 50 years, agricultural research for developing countries has increased production and availability of energy-dense staple crops, but the production of micronutrient-rich non-staples, such as vegetables, fruits, pulses and animal products, has not increased in equal measure. As a consequence, even though staple food prices have declined after controlling inflation, non-staple food prices have increased steadily and substantially, making it more and more difficult for the poor to afford dietary quality (Bouis et al. 2011; Gödecke et al. 2018).

Poor households will typically spend 60-70% of total income on food. Dietary patterns by income group in LMICs show that individuals in poorer and richer households eat about the same amount of total food staples. The first priority in allocating limited food budgets is to keep from going hungry (Bouis 1996).

Animal product consumption (the richest source of bioavailable minerals and vitamins) is generally low in LMICs even in richer households, due to the high cost of animal products per unit of energy (on the order of 20 times as expensive as food staples). Families wish to consume higher amounts of animal products as evidenced by large percentage increases in animal product intake with income, and may spend as much as 30% of food budgets on animal products. Even the diets of higher income households typically do not meet recommended intakes of several micronutrients, especially those for women and children because of their higher requirements (Bouis 1994; Bouis and Haddad 1992; Bouis, Haddad, and Kennedy1992).

Table 1 presents food expenditure and energy and nutrient intake data for the Philippines for 2015, as an example of typical dietary patterns found in most LMICs.[1] The primary basic food staple, rice, is eaten by all income groups in about the same amounts. At the margin as incomes rise, increasing amounts are spent on vegetables and fruits, other cereal processed products (mostly imported wheat in the Philippine context), sugars, fats, and oils, beverages and condiments. However, ***quantities*** for these foods do not rise markedly (as indicated by per capita energy consumption); to a significant extent, consumers are paying for a more desired quality for these types of foods. As incomes rise, the focus is on purchase of animal and fish products, which triple in terms of quantity between lowest income and highest income groups.

Given these patterns of changes in diets with income, Table 1 shows how the percentage contributions to total nutrient intakes change as incomes rise. Clearly, rice contributes the same significant base amount of several nutrients including minerals and vitamins across all income groups, regardless of the extent to which adequacy is achieved. Non-staple foods then add to these base amounts, especially animal and fish products as incomes rise. These base nutrient amounts provided by rice (even if inadequate by themselves) ***relatively*** are far more important to the total nutrient intakes of lower income than higher income groups. Milled rice tends not to be dense in nutrients, but because the quantity eaten is high, multiplying quantity by density, gives a significant absolute intake amount for several nutrients. ***In fact, in the Philippines, no single food in the diet provides more of significant amounts of a broad range of nutrients than rice.***

These same conclusions would apply to other countries where other food staples take the place of rice. The constant across all LMICs is that high amounts of food staples are consumed by all income groups. In many countries, consumers may eat relatively more vegetables and fruits than animal and fish products than is the pattern in the Philippines. However, this only strengthens the point being made here on the importance of food staples as nutrient sources in these other countries, with lower animal and fish product intake, which are the richest, but most expensive source of minerals and vitamins per unit of energy.

In summary, dietary quality improves with income, but only gradually. Incomes of poor households must increase severalfold before dietary quality is adequate, and this is made more difficult by rising non-staple food prices.[2] Significant reductions in national poverty rates can take decades to achieve.

Poverty rates are higher in rural areas (as compared with urban areas), where biofortified crops are first produced and consumed. A key element of the potential effectiveness of biofortification is that because biofortified staple food crops are high-yielding, they will sell for the same price as equivalent non-biofortified products. The value proposition, then, especially to parents, is that substituting biofortified for non-biofortified staple foods provides significant extra minerals or vitamins in family diets at no extra cost. Thus, the following example was given in a recent blog, "if all wheat consumed in Pakistan's Punjab province were zinc-enriched, the cost of a nutritious diet for an adolescent girl would fall by 25 percent" (Brown 2020).

Interventions to Address Mineral and Vitamin Deficiencies and Their Cost

Nutritionists recognized the gap in dietary quality and insufficient micronutrient intakes thirty years ago, beginning with a series of high-dose vitamin A capsule efficacy trials that reduced preschooler mortality by an average of 23% (Beaton et al. 1994). This result for vitamin A led to further investigations for iodine, iron, zinc, and other micronutrient deficiencies.

To date, more than 10 billion vitamin A capsules have been distributed to preschool children over the past 20 years in LMICs, saving millions of lives. More recently, there is a push for universal multiple micronutrient supplementation for preschool children (Tam et al. 2020) and for pregnant mothers to replace iron-folate supplementation (Smith et al. 2017), based on similarly solid evidence produced by much more recent efficacy trials. Commercial food fortification, micronutrient powders, school feeding programs, among other direct nutrition interventions, are also administered widely to reduce or fill people's dietary gaps. With experience and improved technologies, these programs are becoming more efficient over time with an increasing capacity for adding a range of minerals and vitamins in single interven-

[1] It is very unusual to have a published national data set, based on 24-hour recall, that allows analysis of intakes for so many nutrients by income group by food group. The Philippines conducts such surveys once every five years, regrettably a country where no scaling effort has been undertaken to introduce biofortified crops.

[2] See Block et al. (2004) for a convincing analysis of why rapidly rising food price rises in Indonesia (due to a financial crisis) led to an increase in iron deficiency among children

Table 1. Peso Cost, Energy Intake, and Percentages of Total Nutrient Intakes, By Broad Food Group and By Wealth Quintile, Philippines, 2015.

FOOD GROUP/ Wealth Quintile	Per Capita Peso Cost Per Day	Per Capita Energy Intake Per Day	Percentage Contribution to Total Nutrient Intakes Across Food Groups By Wealth Quintile										
			Energy	Protein	Iron	Vitamin A	Calcium	Vitamin C	Thiamine	Ribo-flavin	Niacin	Fats	Carbo-hydrates
ENERGY-GIVING FOODS													
Poorest	21.59	1515	86%	61%	52%	7%	32%	13%	71%	42%	61%	65%	93%
Poor	22.99	1473	82%	55%	51%	9%	28%	7%	65%	38%	57%	57%	92%
Middle	23.77	1452	79%	50%	51%	6%	30%	6%	63%	34%	54%	50%	91%
Rich	26.75	1395	73%	44%	47%	6%	29%	7%	55%	30%	49%	43%	90%
Richest	30.80	1366	68%	38%	42%	5%	27%	8%	48%	28%	43%	39%	88%
BODY-BUILDING FOODS													
Poorest	13.75	150	8%	33%	21%	55%	38%	1%	13%	36%	27%	35%	1%
Poor	19.63	220	12%	40%	26%	59%	50%	4%	21%	44%	33%	43%	2%
Middle	25.85	283	15%	45%	28%	73%	48%	5%	24%	51%	36%	50%	2%
Rich	36.81	401	21%	52%	33%	77%	46%	8%	34%	56%	43%	57%	3%
Richest	50.71	497	25%	58%	38%	78%	51%	8%	42%	61%	48%	61%	3%
BODY-REGULATING FOODS													
Poorest	6.04	61	3%	5%	21%	34%	24%	84%	13%	19%	6%	0%	3%
Poor	6.49	57	3%	4%	17%	25%	18%	84%	12%	14%	5%	0%	3%
Middle	7.13	56	3%	3%	14%	16%	18%	79%	9%	11%	4%	0%	3%
Rich	8.64	59	3%	3%	13%	12%	17%	72%	8%	9%	4%	0%	4%
Richest	11.97	74	4%	3%	13%	12%	16%	67%	7%	7%	4%	0%	5%
MISCELLANEOUS FOODS													
Poorest	3.65	40	2%	1%	6%	4%	5%	2%	3%	4%	6%	0%	2%
Poor	4.19	44	2%	1%	7%	6%	5%	5%	3%	5%	6%	0%	3%
Middle	4.57	52	3%	1%	7%	5%	5%	9%	4%	4%	5%	0%	4%
Rich	5.80	60	3%	1%	7%	5%	7%	12%	3%	5%	5%	0%	4%
Richest	6.81	64	3%	1%	7%	5%	7%	17%	4%	4%	5%	0%	4%
Rice and Rice Products													
Poorest	14.36	1077	61%	46%	37%	0%	22%	0%	47%	28%	53%	9%	72%
Poor	14.70	1077	60%	43%	34%	0%	20%	0%	40%	23%	49%	7%	74%
Middle	14.85	1086	59%	40%	33%	0%	20%	0%	39%	20%	45%	6%	76%
Rich	14.75	1002	52%	34%	28%	0%	20%	0%	30%	17%	39%	2%	74%
Richest	15.61	947	47%	29%	24%	0%	16%	0%	27%	14%	34%	2%	70%
Other Cereals, Roots/Tubers													
Poorest	5.04	304	17%	14%	15%	7%	8%	12%	24%	13%	7%	17%	18%
Poor	5.60	248	14%	11%	16%	9%	5%	7%	24%	14%	9%	13%	14%
Middle	5.98	214	12%	9%	17%	6%	8%	6%	24%	14%	9%	11%	12%
Rich	7.96	226	12%	10%	18%	6%	7%	7%	24%	13%	9%	11%	13%
Richest	10.35	241	12%	9%	17%	4%	11%	7%	21%	14%	10%	9%	14%
Sugar/Syrups													
Poorest	1.05	45	3%	0%	0%	0%	3%	0%	0%	0%	0%	0%	3%
Poor	1.13	44	2%	0%	0%	0%	3%	0%	0%	0%	0%	0%	3%
Middle	1.28	41	2%	0%	0%	0%	3%	0%	0%	0%	0%	0%	3%
Rich	1.74	42	2%	0%	0%	0%	2%	0%	0%	0%	0%	0%	3%
Richest	1.97	40	2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	3%
Fats/Oils													
Poorest	1.14	89	5%	1%	1%	0%	0%	0%	0%	0%	0%	39%	0%
Poor	1.56	104	6%	1%	1%	1%	0%	0%	1%	0%	0%	37%	0%
Middle	1.66	111	6%	1%	1%	0%	0%	0%	0%	0%	0%	33%	0%
Rich	2.30	125	7%	1%	1%	1%	0%	0%	1%	0%	1%	30%	0%
Richest	2.87	138	7%	1%	1%	1%	0%	0%	0%	0%	0%	28%	0%

Source: Food and Nutrition Research Institute, 2015 Dietary Survey, Appendices 55 and 44
Energy-Giving = Rice, Maize, Wheat, Roots & Tubers, Sugar & Syrups, Fats & Oils, Body-Building = Animal & Fish Products, Beans, Nuts, & Seeds, Body-Regulating = Vegetables and Fruits, Miscellaneous = Beverages, Condiments & Spices, Others
Exchange Rate: 47 pesos = US$1.00
Notes: Per capita intake of rice products, expresssed as energy (15 calories per days), accounts for just 1.5% of total rice and rice products intake (1024 calories per day) across all income groups.
Unpublished analysis in progress shows that rice and rice products account for an average percentage contribution (across all income groups) for the following nutrients:

10 of 11 Amino Acids	30-40%	Folate	10%	Vitamin B5	57%	Vitamin B6	32%	Copper	32%
Magnesium	38%	Manganese	53%	Phosphorus	33%	Potassium	15%	Zinc	41%

[the Zinc percentage here is probably understated as the International Master List Food Composition Table (FCT) coefficient for milled rice is 10.6 ppm Zn; Iron percentages in the table above are probably overstated for milled rice as the Philippine FCT coefficient is 10 ppm Fe; HarvestPlus estimated coefficients for unbiofortified milled rice are 16 ppm Zn and 2 ppm Fe] tions, for example adding iron to iodized salt. New fortification interventions are being proposed. Over a period of 10 years, "the cost per death averted through mandatory folic acid fortification is $957 and the cost per disability-adjusted life year is $14.90" (Hoddinott 2018).

A key point is that these interventions involve recurrent annual costs, and do not address the underlying problem of the gap left by agriculture. For example, the 10 billion vitamin A capsules have been distributed at a minimum cost of $10 billion—and still counting. The estimated median cost of adding iron to wheat flour is $0.17 per person per year (Fiedler et al. 2008), while adding iron to iodized salt is a little more costly per person at $0.25 per person per year (Horton et al. 2011). For perspective, then, for one billion people for iron fortification, this is an approximate annual recurrent cost of $200 million per year.

There is an increasing awareness in the nutrition community that a longer-term, more sustainable perspective is required. For example, the headline on the cover of a recent Lancet issue (Volume 395, January 8, 2020) states: "In this nutrition decade, a new global nutrition movement is emerging that needs to take the lead in demanding that ***food systems*** change locally, regionally, and globally. It is within our collective power; we owe it to future generations."

Nevertheless, from an economic perspective there is an inherent budget constraint between relatively expensive direct nutrition investments to avert a negative nutrition outcome in the short-term, and relatively inexpensive agricultural investments that help to solve the underlying problem in the longer-term. The optimal mix of interventions to some extent will be determined by the gestation times and cost-effectiveness of the options available, but fundamentally in each LMIC, there needs to be a short-term strategy and a long-term strategy.

Cost-effectiveness of Biofortification and Other Micronutrient Interventions

Understanding the cost-effectiveness of an intervention is paramount for policy-makers who have to manage a limited budget and prioritize funding for competing interventions. While several interventions may be able to achieve a given set of objectives, the cost of implementing these interventions and of scaling them up may differ. Therefore, a key question is which intervention (or combination of interventions) is the most cost-effective one, meaning that it offers the best value for money (Edoka and Stacey 2020)? No one nutrition intervention can reach all deficient persons and fulfill all nutrient requirements. It is beyond the scope of this section to consider combinations of interventions. Nevertheless, it is informative to compare the cost-effectiveness of biofortification with other common micronutrient interventions.

Different methods and indicators of cost-effectiveness can be used (Stein and Qaim 2007). One widely used approach is to express all negative health outcomes of micronutrient deficiencies—including diseases, physical and mental development impairments, and premature deaths—in terms of disability-adjusted life years (DALYS) and calculate the cost of the intervention per DALY saved (Edoka and Stacey 2020; Stein 2006; Stein et al. 2005).

Over the last 15 years, several studies have used DALYs in cost-effectiveness analyses to quantify the effect of crop biofortification in different countries (e.g., De Steur et al. 2017; Lividini and Fiedler 2015; Meenakshi et al. 2010; Sayre 2011; Qaim et al. 2007; Stein 2010a; Stein 2010b). Most of these studies evaluated biofortification with single micronutrients in specific crops; a few evaluated several micronutrients in the same crop. As the first biofortified crops were only released relatively recently, many of the cost-effectiveness studies evaluated likely future effects (so-called ex *ante* studies) rather than already observed effects (Lividini et al. 2018). What all of these studies suggest is that biofortification can be a highly cost-effective micronutrient intervention, which often costs only a few dollars per DALY saved, far below the World Bank's (2020) threshold of $270 for cost-effectiveness.

Figure 1 shows results from cost-effectiveness studies that were identified through a systematic review. The cost of biofortification includes the cost of breeding, testing, and technology dissemination. For transgenic biofortified crops, estimates of the regulatory costs were also included. Studies are ordered according to the magnitude of their cost-effectiveness estimates. The highly favorable estimates on the left-hand side (low cost per DALY saved, meaning high cost-effectiveness) often build on relatively optimistic assumptions concerning the speed and extent of biofortified crop adoption (Meenakshi et al. 2010; Qaim et al. 2007). For instance, under optimistic assumptions zinc-rich wheat in India can cost as little as $2 per DALY saved, but $40 per DALY saved under less optimistic assumptions (Stein 2010a). The speed and extent of adoption depend on various factors, including the number of biofortified varieties that will be bred and their suitability for various agroecological and socioeconomic conditions, as will be explained in more detail below. But even under less optimistic assumptions, biofortification remains a very cost-effective intervention in most cases (Figure 1).

The main reason for the high cost-effectiveness of biofortification—in spite of substantial breeding and development costs—is that the approach can exploit economies of scale: Once biofortified varieties have been developed, some small additional costs accrue for maintenance and adaptive breeding, but—apart from this—the varieties can spread across time and space due to the self-replicating nature of seeds, thereby multiplying the potential benefits. That is, the positive nutrition and health effects increase at a much higher rate than the costs. Whether planting biofortified crops or not, for farmers nothing changes: apart from the seeds, they use the same inputs, have the same costs, and achieve the same yields and profits.

However, as Figure 1 also shows, biofortification is not highly cost-effective under all possible assumptions. If plant breeders biofortify crops that are not widely eaten in a certain context or that contain too low amounts of bioavailable micronutrients to effectively address widespread deficiencies, the cost per DALY saved can also be above common thresholds for cost-effectiveness (Asare-Marfo et al. 2013; Funes et al. 2015; Stein 2010b). Hence, proper project plan-

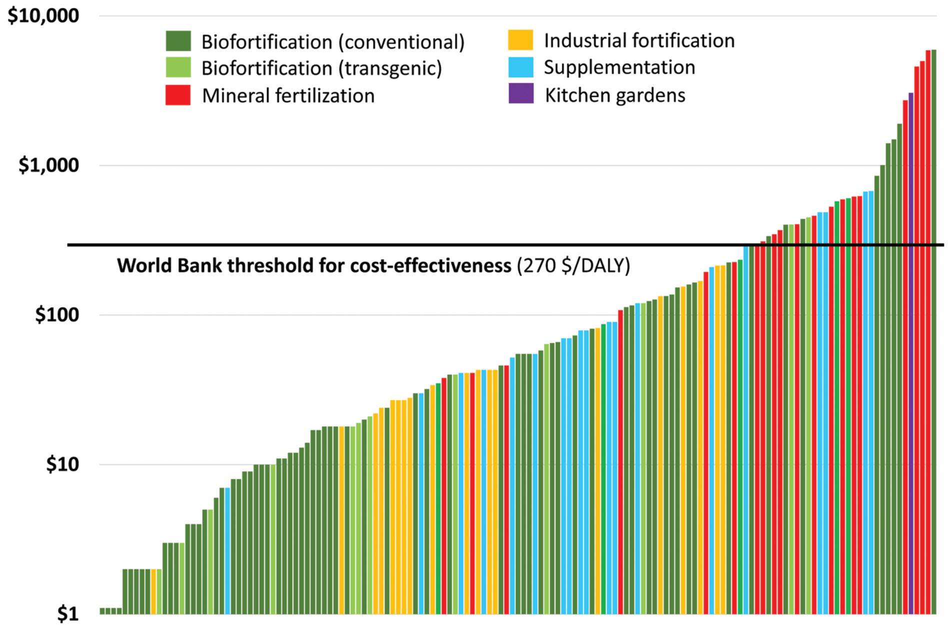

Figure 1. Cost-Effectiveness of Different Micronutrient Interventions. Authors' own presentation based on a large number of original cost-effectiveness studies identified through a systematic literature review. Each column represents one cost-effectiveness estimate expressed in terms of the cost in US$ per disability-adjusted life year (DALY) saved (log scale).

ning and implementation are important, which is true for biofortification as for any other micronutrient intervention.

Interesting in Figure 1 is also the comparison between conventional and transgenic breeding approaches to develop biofortified crops. One the one hand, transgenic approaches could lead to larger positive nutrition and health effects, as they typically achieve higher densities of micronutrients and also facilitate the stacking of multiple nutrient traits in the same crop. On the other hand, given issues with public acceptance, transgenic crops are also associated with much higher regulatory costs than conventionally-bred crops. This is why systematic differences in the cost-effectiveness of both approaches are not expected under current political and societal conditions. Transgenic approaches could become more cost-effective if public acceptance would rise and suitable regulatory reforms were implemented.

Mineral fertilization can also be used to increase the micronutrient content of crops and is therefore sometimes also called "agronomic biofortification" (Joy et al. 2015; Joy et al. 2017; Hurst et al. 2013; Wang et al. 2015; Wang et al. 2016; Zhang et al. 2018). Figure 1 suggests that this can also be a cost-effective micronutrient intervention. However, unlike the breeding approach (conventional and transgenic), there are important recurrent costs involved in "agronomic biofortification", such as the production, distribution, and application of the mineral fertilizer. Mineral fertilization can complement the breeding approach when mineral-deficient soils limit the uptake of minerals by biofortified crops; its cost-effectiveness can be increased if the minerals can get a "free ride" and are applied together with regular fertilizers, Fbut it lacks the economies of scale of the breeding approach.

Also other common micronutrient interventions—such as industrial fortification and supplementation—have recurrent costs, so that their cost-effectiveness is often lower than that of biofortification (Figure 1). However, in general both industrial fortification and supplementation can still be considered cost-effective micronutrient interventions, and they can complement biofortification, as the target populations are not necessarily identical. Whereas biofortification has clear advantages for the rural poor, industrial fortification and supplementation can be more useful for urban households that consume larger quantities of processed foods and tend to have better access to healthcare centers.

More comprehensive nutrition interventions, such as the promotion of kitchen gardens, are less cost-effective than most other micronutrient interventions, mostly because intensive training is needed that can only be provided at smaller scale. In addition, the opportunity costs of the household time and the land needed to establish and maintain the kitchen garden needs to be factored in (Abdoellah et al. 2020; Asaduzzaman et al. 2011; Dragojlovic et al. 2020; Ha et al. 2019; Schreinemachers et al. 2016). On the other hand, kitchen gardens may have a wider range of possible benefits (e.g., improving the intake of more nutrients, empowering women, or increasing marketable production) that are not fully covered in most cost-effectiveness analyses.

Overall, biofortification seems to be a very cost-effective approach—often times more cost-effective than alternative or complementary micronutrient interventions, and even more so than many other public health, nutrition, or agricultural interventions. Therefore, biofortification represents very good value for money.

Key Scientific, Implementation, and Institutional Questions Addressed at the Initiation of Biofortification Research

The analysis in the previous subsection shows that biofortification has a high ***potential benefit***, far exceeding the costs. But *ex ante* analysis is inherently based on a number of assumptions. Initially, there were three primary questions that needed to be addressed—all three questions had to be answered positively for biofortification to be successful.

- When consumed under controlled conditions, will the extra nutrients bred into the food staples be bioavailable and absorbed at sufficient levels to improve micronutrient status (and hopefully also show improved functional outcomes)?
- Can breeding increase the micronutrient density in food staples in high-yielding backgrounds to reach target levels that will have a measurable

Institutional History

Two significant institutional barriers were overcome in successfully initiating biofortification activities. The first issue is the long lag time between investments in biofortification and realizing benefits. Until the last decade, conventional crop development took up to 10 years before the first variety releases occurred. Then significant benefits accrue only when a relatively high percentage of farmers adopt. Can funding be sustained over (say) a 25-year period to prove that biofortification can work? Moreover, after the proof of concept is established, can funding continue to ensure that biofortification is mainstreamed into the fabric of current food systems? The second issue is, if sufficient funding can be developed and sustained, how can the required interdisciplinary and inter-institutional activities be coordinated efficiently to achieve success?

Recognizing the multi-disciplinary, multi-institutional complexity, HarvestPlus began its activities in 2003 as a "Challenge Program" of the Consultative Group on International Agriculture Research (CGIAR), approved by processes put into place by the donors to the CGIAR. HarvestPlus was initially governed as a cooperative agreement between two CGIAR Centers, the International Food Policy Research Institute (IFPRI) and the International Center for Tropical Agriculture (CIAT).

A Program Advisory Committee (PAC) composed of highly respected individuals from a range of disciplinary backgrounds served as a virtual Board An interdisciplinary management staff was hired, some as IFPRI employees (director, nutrition and food science, economics, communications), some as CIAT employees (crop development and genomics, delivery). As of mid-2019, IFPRI is now the single governing Center.

Over seventeen years, HarvestPlus has spent more than $400 million from 35 different donors to undertake the activities described at right.[3]

Consistent progress would not have been possible without sustained and major funding from the Bill and Melinda Gates Foundation (BMGF, about 30% of total funding) and the Department for International Development (DFID) of the United Kingdom (about 30% of total funding). At different points in time, in addition to funding received from A4NH (see footnote 3), the governments of Canada and United States have also made substantial contributions, as have the MacArthur Foundation and the World Bank (collectively about 30% total funding).

In short, with its base in the CGIAR at IFPRI and CIAT, there was the institutional flexibility to receive funds and to write contracts with a range of institutions all over the world (the cumulative count is now over 600).[4] The base in the CGIAR provided the best opportunity to take advantage of the leadership of individual Centers in developing high-yielding staple food crops (on which biofortification "piggybacks") and each Center's long-standing collaborations with the National Agricultural Research Systems (NARS) in multiple countries.

[3] In 2012, in a reorganization of CGIAR activities, HarvestPlus became a subcomponent of one of the CGIAR's broad research programs (or CRPs), Agriculture for Improved Health and Nutrition (A4NH). Some A4NH funding has been directed to HarvestPlus, but HarvestPlus has continued to be governed and managed substantially as before the reorganization. There is reporting to A4NH as to how A4NH funds are spent and outcomes.

[4] This flexibility was less for investments to catalyze crop dissemination. A non-profit, legal entity, HarvestPlus Solutions, has now been established to provide more flexibility for accelerating producer and consumer uptake.

CGIAR Partnerships Under HarvestPlus

Institution	Nutrient and Crop
Primary Investments	
CIAT	iron beans, vitamin A cassava
CIMMYT	vitamin A and zinc maize, zinc wheat
CIP*	vitamin A sweetpotato
ICRISAT	iron pearl millet
IITA	vitamin A cassava, vitamin A and zinc maize
IRRI	zinc rice
Secondary Investments	
Bioversity	vitamin A bananas
CIP	iron potato
ICARDA	iron and zinc lentils
ICRISAT	iron and zinc sorghum
IITA	vitamin A bananas

* CIP vitamin A sweetpotato withdrew from HarvestPlus in 2010. CIP had undertaken activities on vitamin A sweetpotato before the establishment of HarvestPlus.

CIMMYT = Acronym for Spanish, International Center for Maize and Wheat Improvement

IITA = International Institute for Tropical Agriculture

IRRI = International Rice Research Institute

ICARDA = International Center for Agricultural Research in Dry Areas

ICRISAT = International Center for Research in the Semi-Arid Tropics

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Institutional History

continued from page 7

IFPRI's solid standing in the international economics and nutrition communities, and strong emphasis on communications were additional advantages.

Analysis. advocacy and fundraising efforts for biofortification began at IFPRI in 1993, and continued for 10 years before the first large grant from the BMGF was secured in 2003. Thus, prior to the approval of HarvestPlus as a Challenge Program, getting sufficient funding to initiate biofortification had been an uphill and largely unsuccessful battle, although smaller grants had been secured from the government of Demark and the Asian Development Bank, which provided valuable experience and maintained cohesiveness among a small consortium of scientists doing background research (CGIAR Micronutrients Project).

CIP began selecting for improved orange-fleshed sweetpotato varieties in the mid-1990s in collaboration with a few NARS. However, funding activities was a challenge until HarvestPlus provided consistent support from 2004-2009. Due to naturally high levels of beta-carotene, the pre-cursor of vitamin A, being available to exploit in the germplasm, vitamin A sweetpotato emerged as first biofortified crop available for testing for nutritional impact on young children at risk of vitamin A deficiency. A major grant made by the BMGF to HarvestPlus was the financing of the Reaching End Users cost-effectiveness study of an integrated orange-fleshed sweetpotato and community based nutrition education program in Uganda and Mozambique from 2005-2009. Positive findings from that study led CIP as an institution to commit to focusing on developing vitamin A sweetpotato on a larger scale, in Africa in particular.

Building on lessons learned, it was essential to invest in breeding in Africa for Africa, to ensure that relevant agronomic traits and consumer taste preferences were addressed. Again, substantial support from the BMGF ($44 million) enabled key research to be undertaken from 2010-2019 to address remaining bottlenecks to unlocking the potential of vitamin A sweetpotato. Concurrently, CIP launched the broader multi-partner Sweetpotato for Profit and Health Initiative (SPHI) to ensure that improved varieties were disseminated and diversified use of sweetpotato developed and promoted. The SPHI focused on 15 countries, working with over 100 different organizations. Key support for the dissemination effort came from DFID, USAID, and Irish Aid HarvestPlus was a member of the SPHI steering committee, assuring continued knowledge sharing.

Given that the concept of nutrition-sensitive agriculture is widely acknowledged now, it can be hard to imagine how difficult it was to get support within the highly siloed agriculture and health sectors at the beginning of the millennium. The institutional commitments underlying HarvestPlus and CIP enabled an evidence base to be built which is often cited as the best evidence supporting nutrition-sensitive interventions (Ruel et al. 2017).

and significant impact on nutritional status?

- Will farmers grow the biofortified varieties and will consumers buy and eat them in sufficient quantities (especially where there is a color change from white to yellow due to the addition of provitamin A)?

These questions are dealt with sequentially in the following chapters. Suffice it to say that there was strong skepticism in the sectors focused on each of these questions before the evidence was obtained.

Chapter 2.

Bioavailability and Efficacy of Biofortified Crops

One of first challenges confronting proponents of biofortification was to provide rigorous evidence that additional iron, provitamin A carotenoids (PVAC), and zinc in biofortified crops were sufficiently bioavailable to produce a public health benefit. To that end, HarvestPlus and their research collaborators have conducted and published 17 bioavailability and 15 efficacy randomized intervention trials. This chapter builds on the existing published literature by summarizing and discussing the strengths and limitations of the nutritional evidence base for conventionally bred or agronomically biofortified crops.

Iron Biofortification

Bioavailability of iron-biofortified crops

Several plant components such as polyphenols, oxalates, calcium, and phytates can inhibit iron absorption. Most notably, phytates, commonly found in cereals and legumes, tightly bind to inorganic iron rendering it less available for use by the body. Other factors such as genetics, disease states, nutrient interactions and most importantly the host's iron reserves can affect the amount of iron absorbed (Bechoff and Dhuique-Maye 2017).

The bioavailability of iron-biofortified crops was reviewed in 2014 (La Frano et al. 2017) and again in 2017 (Boy et al. 2017). As shown in Table 2, the percent iron absorption for all iron-biofortified or

Table 2. Subject Characteristics and Iron Intake from Iron-Biofortified Staple Food in Four Efficacy Studies.

Crop (Location)	Rice (Philippines)		Beans (Rwanda)		Beans (Mexico)		Pearl millet (India)	
Reference	Haas 2005		Haas 2016		Finkelstein 2019		Finkelstein 2015	
Subject characteristics-total sample	**Adult Females**		**Adult Females**		**Children 5-11 ys**		**Adolescents 12-16 ys**	
Sample size eligible for feeding	192		195		574		246	
Percent anemic (Hemoglobin<120 g/L)	28		36		18		28	
Percent iron deficient (Ferritin<15 μg/L)	34[a]		86		16		43	
Experimental food consumed (g/d)	600		335		67		221	
Iron content of experimental (biofortified and conventional) foods								
	BF	**C**	**BF**	**C**	**BF**	**C**	**BF**	**C**
Iron concentration (mg/kg, dry)	10	2	86	50	94	54	86	21-52
Iron intake from staple (mg/d)	1.8	0.4	13.5	8.0	2.8	1.6	17.6	5.7
Iron intake from experimental crops relative to requirements								
Percent iron absorption[b]	7.3	7.3	7.1	9.2	5.0	5.0	7.4	7.5
Absorbable iron (μg/d)	134	29	1060	791	140	80	1300	428
Physiological iron requirement[c] (μg/d)	1460		1460		800		1170[d]	
Percent daily requirement from staple	9	2	73	54	18	10	111	37

[a] Ferritin <12 μg/L
[b] Iron absorption estimates: Philippines rice based on calculations by Beard et al. 2007; Rwanda beans and Mexico beans based on Petry et al. 2014; pearl millet based on Cercamondi et al. 2013.
[c] Estimated Average Requirement (EAR) of absorbed iron (μg/day), from Institute of Medicine (US) Panel on Micronutrients (2001).
[d] Median EAR for 11–14 year old males, from Institute of Medicine (US) Panel on Micronutrients (2001).

control foods used in the four published efficacy trials was similar to their conventional counterparts (5.0–9.2%) (Cercamondi et al. 2013; Petry et al. 2014). The fractional absorption and use of iron from Indian pearl millet and Rwandan beans was determined by stable isotope methods using a multiple meals and days design (Cercamondi et al. 2013; Petry et al. 2014). The bioavailability of iron in Philippine rice was estimated after the trial using serum ferritin change (Beard et al. 2007).

Efficacy of Iron-Biofortified Crops

Impact on biomarkers of iron status

To date there have been four published efficacy studies with three iron-biofortified crops—rice, beans, and pearl millet—in four different countries (Table 2). Three of these studies have been summarized by Boy and colleagues (2017), and the results were further analyzed in two subsequent meta-analyses (Finkelstein et al. 2017; Finkelstein et al. 2019a). The fourth published study not included in the meta-analyses, was conducted in Mexico with iron-biofortified beans (Finkelstein et al. 2019b).

In all four efficacy studies, free-living human subjects consumed either an iron-biofortified variety of a popular staple food or a control variety of the same food with similar appearance, taste and cooking properties. The populations were selected because surveys indicated high levels of consumption of the staple food crop and iron-biofortified varieties of these crops were available to sustain long-term controlled feeding trials. Participants with elevated risk of iron deficiency were selected and randomly assigned to consume either the biofortified or control variety daily for four to nine months. Iron nutritional status based on serum ferritin (SF), soluble transferrin receptors (sTfR), calculated total body iron (TBI), and hemoglobin (Hb), was assessed at baseline and endline to determine change in status.

The first meta-analysis by Finkelstein and colleagues (2017) pooled the data from the three published feeding trials: rice in Philippine women (Haas et al. 2005), beans in Rwandan women (Haas et al. 2016), and pearl millet in Indian adolescents (Finkelstein et al. 2015). They showed that iron-biofortification improved SF and TBI, both indicators of body iron stores. Not surprisingly, the greatest impact was observed in subjects who were iron deficient at baseline or consumed more biofortified food throughout the respective feeding trials. An increase in Hb was only observed among subjects who were anemic at baseline. When each study was analyzed separately, results for selected high-risk subsamples consistently showed significant effects of consuming the high iron foods on SF and TBI when compared to controls (Table 3). In contrast to the other studies, Hb increased only in the intervention group in Rwanda where most of the anemia was due to iron deficiency. No effect on anemia prevalence was reported.

The second meta-analysis (Finkelstein et al. 2019a) examined the same pooled data looking farther into effects on iron status as categorical (prevalence) outcomes and reported that iron-biofortified foods did not reduce the prevalence of iron deficiency or anemia. The authors suggest that while a significant effect of the interventions was observed when continuous measures of the iron biomarkers are analyzed according to the original efficacy study design, the lack of an effect in categorical measures (which were not intended outcomes of the original efficacy studies) may be due to small sample size or insufficient intervention time.

The most recently published efficacy study was conducted in 574 Mexican primary school children (5–12 years old) residing in 20 rural boarding schools in the state of Oaxaca (Finkelstein et al. 2019b). Because of unanticipated factors, such as unbalanced clusters of infection in control clusters that reduced the interpretability of ferritin results, and teacher strikes and longer holidays that reduced the average number of feeding days from 104 to 68, the study was ultimately underpowered to test for significant intervention effects.

Impact on functional outcomes

Two of the efficacy trials, pearl millet in India and beans in Rwanda, included secondary measures of cognitive and physical performance, both of which are compromised by iron deficiency (Haas and Brownlie 2001; Murray-Kolb 2013; McClung and Murray-Kolb 2013). These outcomes relate to important everyday behaviors and social and economic well-being (Haas and Brownlie 2001; Murray-Kolb 2013; McClung and Murray-Kolb 2013).

Cognitive performance

Cognitive function was tested in both the trials using a similar battery of tests administered to random subsamples from trial participants with lower iron status at baseline. These included computerized tasks of attention, memory and reaction time. Significant improvements in these cognitive domains were observed in both the Rwanda and India studies (Murray-Kolb et al. 2017; Scott et al. 2018). In the Rwandan trial with beans, authors also report significant relationships between improvements in women's iron status (SF, TBI and Hb) and improvements in reaction time measured in a variety of tasks. The treatment effects in each study were strengthened when the data of the two trials were combined in the meta-analysis by Finkelstein and colleagues (2019a).

Physical performance

Physical activity and work efficiency were assessed as secondary outcomes in both the Rwanda and India trials (Luna et al. 2016; Luna et al. 2020). The bean trial with Rwandan university women failed to show a significant treatment effect on physical work efficiency assessed as the energy cost to perform a fixed level of moderate work in a subsample of participants (Luna et al. 2020). However, the authors report statistically significant relationships between changes in iron status resulting from the 128 days of the intervention and work capacity. In women who were anemic at baseline there was a significant relation between increased

Table 3. Comparison of Results from Four Iron Biofortification Efficacy Studies.

Crop (Location)	**Rice (Philippines)**		**Beans (Rwanda)**		**Beans (Mexico)**		**Pearl Millet (India)**	
Reference	Haas et al. 2005		Haas et al. 2016		Finkelstein et al. 2019		Finkelstein et al. 2015	
Participants	**Adult Females**[a]		**Adult Females**[a]		**School Children**[b]		**Adolescents**[b]	
Experimental group	**High iron**	**Control**	**High iron**	**Control**	**High Iron**	**Control**	**High iron**	**Control**
Sample size[c]	69	69	94	101	269	305	98	95
ΔHemoglobin (g/L)	1.1	0.9	2.8 [d]	-1.0	0.0	0.1	1.3	0.9
ΔFerritin (*μ*g/L)	1.1 [d]	-4.27	5.50 [d]	3.60	4.83	8.03	5.7 [d]	1.2
ΔTransferrin receptor (mg/L)	0.35	-0.15	-0.10	-0.20	-0.05	0.11	0.21	0.33
ΔBody iron (mg/kg)	0.63 [d]	-0.25	1.40 [d]	0.90	0.77	0.67	0.83 [d]	0.02
Subsample description	Non-anemic (Hb>120 g/L) at baseline		Low ferritin (<20 *μ*g/L) at baseline		All enrolled children (16% iron deficient and 18% anemic)		Low ferritin (<20 *μ*g/L) at baseline	

Values are change (Δ) in iron status indicator from baseline to end line.

[a] Difference in mean values

[b] Males and females combined, difference in median values

[c] For indicated subsamples

[d] Significant difference between iron-biofortified and control groups, $p<0.05$

Hb and reduced energy cost to perform a moderate level of physical work. In non-anemic women at baseline, an increase in SF was significantly related to improvement in the measure of work efficiency (Luna et al. 2020).

In the pearl millet trial with Indian adolescents, physical performance was assessed through measure of physical activity determined over six days by accelerometers (Luna et al. 2016). Children who consumed iron-biofortified pearl millet logged significantly more light physical activity and fewer minutes of sedentary time each day compared to those who consumed the control variety (Luna et al. 2016). Additionally, the amount of iron consumed per day over the course of the trial was directly related to minutes spent in light physical activity and inversely related to daily sedentary minutes.

Provitamin A Biofortification

Bioconversion efficiency of provitamin A biofortified crops

To contribute to vitamin A (VA) activity, PVAC—β-carotene (βC), α-carotene, and β-cryptoxanthin—must be absorbed and converted to VA in the body. Numerous factors along this pathway can impact the population and individual response to PVAC, including crop processing and storage, cooking methods, food matrix and other ingredients included in the meals (e.g., lipids), baseline VA status, and genetics (Haskell et al. 2004).

Absorption studies have demonstrated that PVAC are both absorbed and converted to VA (Li et al. 2010; Muzhingi et al. 2011; Titcomb et al. 2018). Absorption and bioconversion studies with orange maize have demonstrated favorable PVAC to retinol conversion ratios ranging from 3:1 to 7:1 (Li et al. 2010; Muzhingi et al. 2011) and among women in the United States, biofortified cassava demonstrated absorption and conversion rate of 4:1 (µg βC to µg RAE) (La Frano et al. 2013). When prepared as commonly consumed *gari*, biofortified cassava increased circulating VA, βC, and α-carotene (Zhu et al. 2015).

Efficacy of Provitamin A Biofortified Crops

Multiple biomarkers are used to investigate VA status and to determine the efficacy of PVAC interventions (Tanumihardjo et al. 2016). Two major categories of biochemical indicators are direct measurement and dose-response, and there is variation in biomarker uses, advantages, considerations, and impact of inflammation (Table 4).

Table 4. Biochemical indicators of vitamin A status: Advantages, considerations, and impact of inflammation.

Biomarker	Advantage	Consideration	Impact of Inflammation
Direct Measurement			
Serum or plasma retinol (SR)	Widely used biomarker	Homeostatically controlled, may only respond in severe deficiency	Reduced during inflammation
Retinol-binding protein (RBP)	Easier/cheaper to quantify than SR	Homeostatically controlled, may only respond in severe deficiency	Reduced during inflammation
Breast milk retinol	Less invasive than blood Reflect maternal VA status and child VA exposure	May be more reflective of recent intake over long-term status Limited to populations able to provide breast milk	Possibly reduced with inflammation
Serum or milk carotenoids	Reflect specific carotenoid exposure	Can be confounded by relative conversion of PVAC to VA	
Dose-Response			
Relative dose response (RDR)	Determines if stores are sufficient or deficient	Requires VA dose and two blood samples	Susceptible to inflammation
Modified relative dose response (MRDR)	Determines if stores are sufficient or deficient	Requires VA analog dose and one blood sample	Susceptible to inflammation; MRDR value may not vary as much as RDR with inflammation
Total body stores (TBS) by retinol isotope dilution (RID)	Quantitative estimate of total body VA stores	Requires a labelled VA dose, one or two blood samples, and technical analysis	Assumptions for calculating TBS impacted requiring modification (e.g. dose absorption, body partitioning, metabolism)

Notes: PVACs=provitamin A carotenoids, VA=vitamin A

Sweetpotato

Studies evaluating high-PVAC orange sweetpotato (OSP[5]) have ranged from controlled feeding efficacy studies to randomized effectiveness trials (Table 5). Randomized controlled effectiveness trials in Uganda and Mozambique increased OSP, PVAC, and VA intakes (Hotz et al. 2012a;b). There was also an observed reduction of diarrhea prevalence among children under five (11.4%) and children under three (18.9%) in the OSP group in the Mozambique trial (Jones and de Brauw 2015). Additionally, a follow up trial conducted three years after the completion of the Mozambique study showed a significant increase in VA intakes among women and children (6–35 mo)—who were born after the completion of the pilot study—in the original OSP households compared to control households (De Brauw, Moursi, and Munhaua 2019). These results highlight the lasting effects of the OSP intervention.

Changes in total body stores in response to OSP feeding was determined in controlled feeding trials among men (Haskell et al. 2004) and nonpregnant, nonlactating women (Jamil et al. 2012) in Bangladesh. In men, change in VA total body stores (TBS) had a mean positive estimate, which was between VA+ and VA- control groups, and not significantly different from either. In women, VA TBS increased in all groups including VA- and VA+ controls but did not differ among groups. A conversion factor of 13.4 (µg βC : µg VA) was determined (Haskell et al. 2004), similar to the factor of 12 used to determine RAEs for βC in foods (Institute of Medicine (US) Panel on Micronutrients 2001).

A controlled feeding trial with children 5–10 years old in South Africa demonstrated increased VA stores following OSP consumption using the modified relative dose response (MRDR) assay (van Jaarsveld et al. 2005). Some studies have demonstrated improvements in serum or plasma retinol over time (Jamil et al. 2012; van Jaarsveld et al. 2005; Low et al. 2007a; Turner et al. 2013) or when compared to a VA- control group over time (Haskell et al. 2004; Low et al. 2007b). In some instances, serum retinol (SR) did not change among a broader group of participants, but the prevalence of SR < 1.05 µmol/L was reduced by 9.5% in a subset of children with adequate data that were controlled for confounding of inflammation, age, deworming, and VA supplementation (Hotz et al. 2012b).

In lactating women in Bangladesh, breastmilk βC increased over time in the OSP group when expressed as overall concentration or per unit of milk fat. Breastmilk VA equivalents for the OSP group was between estimates for the VA- and VA+ groups, and not significantly different from either (Tuner et al. 2013).

Pupillary threshold improved among OSP and control groups over time; groups that consumed boiled or fried OSP were between effects for VA- and VA+ groups, and not statistically differ from either (Jones and de Brauw 2015).

Maize

High PVAC orange maize has been evaluated with longer-term RCTs on biochemical and functional indicators of VA status (Table 6). Among preschool children in Zambia, consumption of high PVAC orange maize significantly improved VA TBS over time compared to a control group who consumed white maize, and was not significantly different from a VA+ group (Gannon et al. 2014).

Among three other trials in children and lactating women in Zambia, orange maize was readily consumed by participants and increased serum βC, α-carotene, β-cryptoxanthin, and zeaxanthin (Palmer et al. 2018; Palmer et al. 2016b; Sheftel et al. 2017; Palmer et al. 2016). In these trials, breast milk or SR did not differ significantly among groups; however, orange maize elevated the natural abundance of ^{13}C in SR, indicating that the orange maize was contributing to body VA stores (Sheftel et al. 2017). MRDR values indicated loss of VA stores in orange and white maize groups after receiving high-dose VA supplementation (Bresnahan et al. 2015).

Pupillary responsiveness was also improved among children who consumed orange maize compared to white maize in children with marginal VA status (SR <

[5] Orange refers to the color of the flesh of the sweetpotato, referred also in the literature as orange-flesh sweetpotato (OFSP).

Table 5. Efficacy of orange sweetpotato.

Reference	Location
Haskel et al. 2004	Bangladesh
van Jaarsveld et al. 2005	South Africa
Low et al. 2007b	Mozambique
Jamil et al. 2012	Bangladesh
Turner et al. 2013	Bangladesh
Hotz et al. 2012b	Uganda
Hotz et al. 2012a	Mozambique
Jones and de Brauw 2015	Mozambique

Notes: AGP=alpha-1-acid glycoprotein
RCT=randomized controlled trial

Study	Feeding Duration	Population, Age	Daily Intake	n (group)	Outcome over time	Outcome relative to control
RCT	60 d	Men, 15-35 y	160 g OSP, 4.5 mg βC	14 (VA-) 14 (OSP) 14 (VA+)	SR constant βC increased TBS positive mean estimate	SR elevated βC increased TBS between estimates of VA- and VA+ controls, not different from either
RCT	53 d	Children, 5-10 y	124 g OSP, 12.4 mg βC	89 (VA-) 89 (VA+)	SR increase in OSP, VA-	MRDR: improved VA status SR similar
RCT (cluster)	2 y	Children, mean age 13 mo at baseline	2.0 mg βC	243 (VA-) 490 (OSP)	VA intakes increase Prevalence of low SR decrease	VA intakes increase Prevalence of low SR decrease
RCT	60 d	Women (nonpregnant, nonlactating), 18-45 y	128 g OSP, ~12 mg βC	30 (VA-) 30 (OSP boiled) 30 (OSP fried) 30 (VA+)	SR increase except boiled OSP group βC increased in OSP groups Pupillary threshold improved in all groups TBS increase in all groups	SR in VA+ > boiled OSP; otherwise no other differences. βC increased in OSP Pupillary threshold in OSP groups not different from either VA- or VA+ groups. TBS not different by group
RCT	3 wk	Women (lactating), 18-45 y	100g OSP, ~12 mg βC	33 (VA-) 34 (OSP) 34 VA+	SR increase in OSP, VA+ groups Serum βC increase with OSP Milk VA concentration similar Milk VA/ fat: increase in VA+, decrease on VA-, OSP.	SR in VA+ > VA-, OSP group not different from either. Serum βC higher in OSP group Milk VA concentration: VA+> VA-; OSP group not different from either Milk VA/fat: increase in VA+, decrease in VA-, OSP
RCT; effective-ness	2 y	Women and children, Children 6-35 mo, 3-5 y		510 (intensive OSP program), 328 (reduced OSP program), 509 (control)	OSP and VA intakes: increased in both intervention groups Reduced prevalence of SR < 1.05 in subset of children with complete data on confounders. Similar SR among children with only SR, CRP, AGP Similar SR among women	OSP and VA intakes: increased in both intervention groups relative to control
RCT; effective-ness	Intensive program: 3 y Reduced program: 1 y	Women and children 6-35 mo, 3-5 y	Mothers: 144-165 g OSP Children 6-35 mo: 47-56 g OSP Children 3-5 y: 73-81 g OSP	265 (intensive OSP program), 255 (reduced OSP program), 259 control	OSP and VA intakes: increased in both intervention groups Reduced prevalence of inadequate intake in intervention groups Among both age groups of children and women	OSP and VA intakes: increased in both intervention groups relative to control Reduced prevalence of inadequate intake in intervention groups relative to control Among both age groups of children and women
Cluster-randomized impact evaluation	~2.5 y	Children		184 (Control) 369 (OSP)		Reduction of diarrhea prevalence and duration

βC=β-carotene CRP=C-reactive protein MRDR=modified relative dose response OSP=orange sweetpotato
SR=serum retinol TBS=total body stores VA=vitamin A VA+=vitamin A positive control group VA-=negative control group

1.05 µmol/L) at baseline (Palmer et al. 2016c)

Cassava

Two efficacy trials with PVAC cassava have been completed, each of which used cultivars with only 50-60% the full PVAC target concentration of 15 ppm. A randomized controlled efficacy study among 5–13-year-old children in Kenya found that SR concentrations in the biofortified group were maintained over time and 0.05 µmol/L higher than the control group, while serum βC was increased with consumption of biofortified cassava (Talsma et al. 2016). A randomized controlled efficacy study in Nigeria with 159 children (3–5 years old) compared the effect of consuming meals made with either standard white (control) or biofortified (yellow) cassava (containing ~9µg/g β-carotene at maturity) twice a day for 16 weeks under direct supervision in study daycare centers. Adjusted SR and Hb concentration adjusted to for malaria infection were modestly but significantly increased (SR: 0.06 µmol/L; 95% CI: 0.004, 0.12; Hb: 3.08 g/L, 95% CI: 0.38, 5.78) in children who consumed biofortified cassava compared to control (Melse-Boonstra et al. in press).

Zinc Biofortification

Absorption of zinc from biofortified crops

Factors recognized to affect zinc absorption include the amount and form of zinc consumed; dietary promoters, such as animal protein; and dietary inhibitors, most notably phytate, which severely limits the net amount of zinc accessible for absorption; and physiologic states, such as pregnancy, lactation and early infancy, all of which increase the demand for absorbed zinc (Krebs 2000; EFSA 2014).

Human stable isotope studies are the gold standard to assess the fractional absorption of zinc (FAZ, %) and estimate total absorbed zinc (TAZ, mg/d[6]). A 2014 systematic review of zinc absorption studies was completed by La Frano and colleagues. The human isotope studies included in this review indicate that the TAZ from biofortified crops (wheat and pearl millet) is significantly higher than from their conventional counterparts (an additional 0.3–0.5 TAZ), provided a threshold nutrient content (i.e., target level) is preserved until the time of consumption (Kodkany et al. 2013; Rosado et al. 2009).

Research has also confirmed that absorption from biofortified and non-biofortified food is inversely proportional to the phytate content of the meals that supply the zinc and directly proportional to the severity of processing (e.g., level of milling) and cooking methods, which increase phytate degradation (Islam et al. 2013; Rosado et al. 2009)

Since the La Frano and colleagues (2014) review, there have been three additional human isotope studies published with three different zinc biofortified crops: maize, rice and wheat. Zinc absorption from maize porridge (biofortified, conventional, and post-harvest maize) was compared in young children (17–44 months) (Chomba et al. 2015). FAZ was similar between the biofortified (22%) and conventional maize (28%) and between biofortified and post-harvest fortified maize, but significantly lower for the post-harvest fortified maize (20%) compared to conventional maize (28%). Children who consumed biofortified and fortified maize absorbed significantly more zinc than children who consumed the conventional maize (an additional 0.6 and 0.5 TAZ, respectively).

Zinc absorption from biofortified (hydroponically enriched) and post-harvest fortified rice was measured using extrinsic isotope labels (Brnić et al. 2016). FAZ for the biofortified variety was 25% and was similar to the post-harvest fortified rice.

Lastly, zinc absorption from 80% and 100% extracted wheat flour (agronomically biofortified, conventional, and post-harvest fortified) was compared in healthy adult women (Signorell et al. 2019). FAZ was lower for the biofortified versus conventional wheat at 80% extraction but did not differ at 100% extraction. Regardless, the TAZ was significantly increased with biofortification because more total zinc was provided with the biofortified wheat flour compared to the conventional wheat flour (7.54–10.06 mg/d vs 4.96–6.54 mg/d). For both extraction levels, biofortified wheat flour provided at least 40% more TAZ than the conventional wheat flour, and a similar amount to the post-harvest fortified wheat flour. Thus, this study confirms that significantly more zinc is absorbed from biofortified wheat compared to conventional wheat varieties.

[6] Total absorbed zinc (TAZ, mg/d) is calculated by multiplying the fractional absorption of zinc (FAZ, %) by the total dietary zinc (mg/d).

Table 6. Efficacy of provitamin A maize.

Reference	Location
Li et al. 2010	USA
Muzhingi et al. 2011	Zimbabwe
Bresnahan et al. 2014	Zambia
Sheftel et al. 2017	“
Gannon et al. 2014	Zambia
Palmer et al. 2016	Zambia
Palmer et al. 2018	“
Palmer et al. 2016	“
Palmer et al. 2016	Zambia
Titcomb et al. 2018	USA

Notes: βC=β-carotene βCX=β-cryptoxanthin
VA+=vitamin A positive control group

Study	Feeding Duration	Population, Age	Daily Intake	n (group)	Outcome over time	Outcome relative to control
Single test meals, random crossover	Single meal	Women, 18-30 y	250 g OM porridge, 0.5 mg βC	6 (crossover; OM, βC+, VA+)	OM βC absorbed and converted to VA	OM VA equivalence: 6.5 μg βC to 1 μg RAE
Single test meals, OM followed by VA+	Single meal	Men, 40-70 y	300 g OM porridge, 1.2 mg βC	8 (paired OM, VA+)	OM βC absorbed and converted to VA	OM VA equivalence: 3.2 μg βC to 1 μg RAE
RCT	70 d	Children, 2-5 y	~250-260 g OM porridge,	95 (OM) 86 (VA-)	MRDR indicate reduced VA stores over time following high-dose VA supplementation	MRDR not different between groups
"	46 d	"	"	45 (OM) 43 (VA-)		Increased serum βCX, lutein, and zeaxanthin Increased 13C in SR
RCT	90 d	Children, 5-7 y	~240 g OM porridge (1x/d), ~280 g OM stiff porridge (2x/d) 2.9 mg βC	44 (OM) 44 (VA-) 45 (VA+)	TBS increase on OM, VA+ groups SR not different across time	TBS of OM, VA+ groups not different and higher than VA- SR not different among groups
Cluster RCT	6 mo	Children, 4-8 y	~ 150 g OM dry weight	543 (OM) 481 (VA-)	SR not different	SR not different Serum βC higher with OM
"	"	"	"	358 (OM) 321 (VA-)		Serum βC, α-carotene, β-cryptoxanthin, and zeaxanthin higher with OM
"	"	"	"	134 (OM) 138 (VA-)		Greater improvement in pupillary responsiveness among children with SR < 1.05 μmol/L
RCT	3 wk	Breastfeeding women, 18-35 y	~260 g OM dry weight	48 (OM) 45 (VA-) 47 (VA+)		Plasma βC higher with OM SR not different among groups Breast milk retinol and βC not different among groups
RCT; crossover	12 d/treatment	Adults, 20-28 y	0.5 mg βCX 0.3-0.4 mg βC 0.8-0.9 mg zeaxanthin	9 (crossover)	Serum βCX and zeaxanthin increased	Serum βCX and zeaxanthin higher 13C in SR not different among groups

OM=orange maize RAE=retinal activity equivalents RCT=randomized controlled trial SR=serum retinol VA=vitamin A
VA-=negative control group

Efficacy of zinc-biofortified crops

One published study examined the efficacy of agronomically biofortified wheat on the zinc status of mothers and their young children (Sazawal et al. 2018). Three other randomized controlled studies (one rice efficacy and two wheat effectiveness) are either in-progress or completed, but unpublished (Ohly et al. 2019; U.S. National Library of Medicine 2019a; U.S. National Library of Medicine 2019b).

The published biofortified wheat study was a community-based RCT conducted in two urban slums in Delhi, India with 6,005 mother (15–49 years) and child (4–6 year) pairs (Sazawal et al. 2018). Pairs were randomly assigned to receive either whole grain flour from a single commercial wheat variety that had either been agronomically biofortified or grown without zinc fertilizer (control). The biofortified whole wheat flour contained an average of 9.75 ppm more zinc than the control flour. Flour was delivered to the household every 25 days over six months, with instruction for the mothers to consume 360 g and their child to consume 120 g daily as bread or porridge. Additional flour was provided to other family members to avoid dilution of the intervention.

After the six-month feeding period, there were no significant treatment effects for plasma zinc concentration (PZC) in either the mothers or children. There were, however, significant differences in reported morbidities: mothers receiving zinc wheat reported significantly fewer days with fever and children receiving zinc wheat reported significantly fewer days with pneumonia and vomiting.

There are, important gaps that limit the interpretation of these findings, even though most of the proposed issues should have been avoided by randomized allocation to study groups. First, inflammation status of the women and children was not measured beyond weekly clinical assessment by morbidity recall questionnaire. Second, the average difference in zinc content between treatments (10ppm) was lower than intended (20ppm). Lastly, authors did not properly control, assess or report how much flour was consumed and how much zinc and phytate were provided in the diet for both groups. These aspects can only be inferred from compliance data and the concentration of zinc and phytate measured in pooled samples from each batch of flour. Back-of-envelope calculations from the compliance data suggest that mothers consumed approximately 280 grams of flour per day and children consumed approximately 90 grams of flour per day, approximately 30% less than intended.

Conclusions for Bioavailability and Efficacy Studies

Studies with iron, PVAC and zinc-biofortified crops have consistently shown that (1) these nutrients are absorbed in significantly greater quantities than their non-biofortified counterparts, (2) PVACs from biofortified crops are efficiently converted to VA by humans, (3) consumption of biofortified crops can significantly contribute to women's and children’s physiological requirements for iron, VA and zinc, and (4) iron and VA stores can be significantly improved when the biofortified foods are consumed as the main staple food.

The RCTs conducted over 17 years constitute predominantly Grade ‘A’ level evidence and represents an evolution and refinement of methodologies that progressively strengthened the case for biofortification. Further, the efficacy trials demonstrate that increases in iron, PVAC, and zinc intakes from biofortified foods result in improved functional outcomes along the health spectrum of undernutrition, in both women and children. Specifically, iron-biofortified beans and iron-pearl millet improved cognitive and physical performance and OSP and zinc wheat reduced morbidity. This provides additional justification to support biofortification as an efficacious strategy to reduce micronutrient deficiency—and the disease and health burden associated with it—in vulnerable populations.

While not all studies demonstrated consistent effects across crops and biomarkers, there are several aspects of processing, storage and micronutrient biology that should be considered when evaluating evidence or planning future directions for biofortified crops (Haskell et al. 2004; Suri and Tanumihardjo 2016; Díaz-Gómez 2017). These include the need to consider PVAC degradation during storage and loss of minerals during processing in real world settings, the safety of consumption of PVAC biofortified crops relative to foods fortified with preformed VA, and the variability and limited sensitivity of biomarkers, especially for zinc. Improving our understanding of nutrient metabolism, biomarkers, and impact of inflammation will help interpret results from previous studies and guide future work evaluating biofortified crops. Developing and standardizing more sensitive biomarkers of nutrient status to reduce cost and technical demand could improve assessment availability.

Future Research Directions

Biomarkers

A challenge to the efficacy trials is the different performance of available nutrient-specific biomarkers in consistently and accurately measuring the effects of interventions on nutritional status. This is particularly true for vitamin A and even more so for zinc studies. Widely used indicators for assessing population zinc and vitamin A status, such as PZC, SR and RBP, are under strong homeostatic control and may only decline when inadequate intake of these nutrients are severe or prolonged (Hess et al. 2007; Tanumihardjo et al. 2016). In addition, interpretation of commonly used biomarkers for iron, zinc, and vitamin A are complicated by the presence of inflammation, which can directly impact measured biomarkers or physiological assumptions used for dose-response tests, in the case of vitamin A (Raiten et al. 2015; Suchdev et al. 2016). For example, during inflammation and infection PZC rapidly declines, in part to deprive microorganisms of zinc, protecting the host and bolstering its ability to fight infection through various intracellular mechanisms (King et al. 2016). Thus, determination of and adjustment for inflammation in study participants and populations is critically important to describe the inflammatory burden’s impact on nutritional biomarkers.

To overcome some of these challenges, there has been recent advancements in identifying novel sensitive biomarkers to asses changes in dietary zinc intake, namely DNA strands damage as measured by the Comet Assay (Zyba et al. 2017); and a decrease in plasma FADS1 activity that lead to a decrease in arachidonic acid levels (Suh et al. 2017). Validation of the field-friendlier option, FADS1 activity, is underway using samples from women and children that participated in biofortification efficacy trials in Pakistan and Bangladesh, respectively.

Improving micronutrient bioavailability

Greatly improving iron and zinc bioavailability in staple crops would significantly increase the impact of achievable biofortification target levels and/or decrease the levels that plant breeders need to reach (Kodkany et al. 2013). This is because of the presence of certain compounds, including phytates and some polyphenols, in these foods that limit people’s ability to digest and absorb these minerals (Beasley et al. 2020; Zheng et al. 2010). Reducing phytate and certain polyphenols in plant foods is a possibility; however, many of these compounds perform beneficial functions in humans and plants. For example, phytic acid has been shown to attenuate iron-induced lipid peroxidation in the colon, decrease kidney stone risk, and osteoporosis risk (Petroski and Minich, 2020).

Importantly, there are also compounds that can promote or enhance iron and zinc bioavailability in plant foods. For example, in vitro and animal studies show that nicotianamine, a non-protein amino acid, can promote iron bioavailability in crops containing high levels of phytate (Bouis and Welch 2010; Wilson et al. 2020). Plant breeding efforts should include increasing the levels of promoter substances in staple plant foods. More research should be done to identify additional promoter substances in plant foods that could be targeted through plant breeding efforts. Genetic engineering is also an important tool to use to increase promoters in staple plant foods.

Prebiotics

Interest in the role prebiotics (and associated probiotics) play in nutrition and health has rapidly grown in the last decades. Beneficial bacteria have been shown to have profound effects on human health (Kennedy, Nantel, and Shetty 2003). Regular consumption of prebiotics that promote beneficial gut bacteria can reduce gut inflammation, which will benefit the absorption and utilization of nutrients. Many biofortification target populations are plagued with gut inflammation for a variety of reasons, including gut parasites, diseases, and diets that are increasingly reliant on ultra-processed foods. Lowering gut inflammation in these populations would greatly improve their ability to absorb nutrients from their diets, particularly iron. Therefore, increasing the levels of prebiotics should become a screening objective for biofortification (UNICEF Micronutrient Initiative 2009).

Need for additional micronutrients

There are significant numbers of people worldwide suffering from iodine and selenium deficiencies as well as deficiencies of several B-vitamins. Interestingly, the root cause of iodine and selenium deficiencies in many regions is insufficient iodine and selenium in cultivated soil to allow enough accumulation in agricultural products to meet human needs (Lyons 2018; Miller and Welch 2013). Deficient soils need to be modified in ways that will provide more available iodine and selenium to food crops. For this reason, agronomic biofortification, in addition to plant breeding, should be recommended in these locations. This includes using fertilizers, either in soil, irrigation water, or foliar sprays, in the right form, right amount, right time, and right place (Lyons 2018; Lyons and Cakmak 2012). Importantly, only relying on plant breeding or using direct fortification (e.g., iodized salt) or supplementation interventions will never sustainably resolve iodine and selenium deficiencies in food systems deficient in these micronutrients.

Not only do humans suffer from deficiencies of these minerals but also livestock and other animals in the food system, which reduces animal productivity (Cao et al. 1994). This greatly lowers farmer income because livestock are important sources of revenue. Reduced income limits resource-poor farmer families' ability to diversify their diets and acquire enough micronutrient-dense foods to meet their needs. Therefore, it is important to correct iodine and selenium deficient soils through agronomic biofortification in addition to plant breeding programs.

CHAPTER 3. Crop Development

The initial question facing plant breeders was could high iron, zinc, and vitamin A density be combined with high yields and profits? While traditional crop improvement focuses on value added traits for existing markets that provide superior crop and/or marketing options to farmers, biofortification breeding adds traits which positively affect human health to these product profiles. This entails integration of nutrition in setting target levels or defining standards based on the likely contribution of these traits to nutritional status in defining trait values. In parallel, coordinated marketing research needs to be conducted in order to assure that requirements and value propositions for all value chain actors are incorporated in product profiles. Once early proof-of-concept research confirmed the feasibility that breeding can add the required nutrient target levels to staple crops, breeding for micronutrient density assumed full operational scale under HarvestPlus during 2007–2010. Initial and progressive waves of biofortified varieties now are planted by more than 10 million farmers in more than 30 countries.

The crop development process entails screening germplasm for available genetic diversity, pre-breeding parental genotypes, developing and testing micronutrient-dense germplasm, conducting genetic studies, applying rapid generation advance through "speed breeding" and developing and applying molecular markers and genomic selection strategies to lower costs, accelerate pace, and boost rates of genetic gain. Once promising lines or hybrids are developed, they are tested in numerous locations across target environments to assess genotype x environment interaction (GxE)—the influence of the growing environment on micronutrient levels, agronomic performance and end-use traits of the varieties or hybrids tested. Large scale regional GxE testing now enables reduced time-to-market for biofortified varieties by using spatial environmental variation to substitute for temporal variation, and eliminating testing steps.

Setting Targets

Early in the conceptual development of biofortification, a working group of nutritionists, food technologists, and plant breeders established nutritional breeding targets by crop, based on food consumption patterns of target populations, estimated nutrient losses during storage and processing, and nutrient bioavailability (Hotz and McClafferty 2007). As with commercial fortification, breeding targets for biofortified crops were designed to meet biologically important proportions of the specific dietary needs of women and children. The answer is complex, depending on age- and gender-specific nutrient requirements, per capita consumption of a particular food, bioavailability of the nutrients, and nutrient retention—as shown in the equation on page 18.

Table 7 shows the target increments

for plant breeding and the incremental percentages of the EAR provided by biofortified crops for preschool children 4–6 years old and for non-pregnant, non-lactating women of reproductive age.

In addition to direct breeding for higher nutrient levels, genotypic differences in bioavailability and retention offer potential to contribute to effectively achieving targets through indirect breeding if genetic variation and prerequisite criteria to address these relevant complex traits through breeding exist. Breeding for micronutrient bioavailability per se is greatly limited by the lack of large-scale, rapid in vitro and/or animal bioavailability models for germplasm evaluation. Hence, dissecting overall bioavailability into its causal components and selectable traits, such as anti-nutrients and promoters, is a common current approach.

Measuring Micronutrient Densities

Existing genetic variation, trait heritability, gene action, associations among traits, available screening techniques, and diagnostic tools are commonly considered and used to identify selectable traits and estimate potential genetic gains through breeding. Biofortification breeding required developing or adapting cost-effective and rapid high throughput analytical techniques for micronutrients, as thousands of samples need to be tested for mineral or vitamin content each season. In parallel with screening, inexpensive analytical methods for high throughput micronutrient screening were tested and further developed along with non-contaminating milling and grinding equipment to boost breeding effectiveness (Yasmin et al. 2014). Contamination in mineral analyses—for example, by iron—resulting from soil, dust, or threshing equipment can be detected using indicator elements aluminum, titanium, and chromium that that are abundant in nature, but absent or present only in trace amounts in plants or seed. Factors that pose challenges to sampling and trait diagnostics are short sample analysis turnaround requirements for crops with two or more cycles per year and rapid post-harvest deterioration, particularly tuber crops or fruits, which are harvested with high moisture content. In contrast to minerals which are very stable, provitamin A carotenoids undergo significant degradation during storage, drying, milling, and processing.

Next to precision analysis methods for minerals (such as Inductively Coupled Plasma Argon Optical Emission Spectrometer; ICP), the high throughput X-Ray Fluorescence Spectrometer (XRF) method has been adapted to analysis of plant samples (Guild et al. 2017; Guild and Stangoulis 2016; Paltridge et al. 2012a; Paltridge et al. 2012b; Sosa et al. 2018). XRF requires minimal pre-analysis preparation and allows for non-destructive analysis and was widely implemented. Due to its sensitivity and

Equation. Biofortification of crops variables.

$$\frac{\text{Extra Nutrient Supplied Through Biofortification}}{\text{Nutrient Requirement}} = \text{Additional Percentage of Estimated Average Requirement Supplied}$$

where Extra Nutrient Supplied Through Biofortification =

$$\text{Per Capita Consumption of Food Staple} \times \text{Increment in Density of Mineral/Vitamin Due to Plant Breeding} \times \text{Percent Retention of Mineral/Vitamin in Processing/Storage/Cooking} \times \text{Percent Bioavailability of Mineral/Vitamin as Consumed}$$

Table 7. Target increments for plant breeding.

Crop	Target Increment	% Target Increment in Released Varieties	Estimated Proportion of the Estimated Average Requirement Provided for Non-Pregnant, Non-Lactating Women		Estimated Proportion of the Estimated Average Requirement Provided for Children, 1-6 years old	
			Before Biofortification	After Biofortification	Before Biofortification	After Biofortification
Beans	+44 ppm iron	50 - 100%	50%	90%	40%	75%
Cassava	+15 ppm pro-vitamin A	50 - 75%	0%	≥100%	0%	95%
Maize	+15 ppm pro-vitamin A +12 ppm zinc	50 - 75% 50 - 75%	0% 45%	55% 70%	0% 55%	60% 80%
Pearl millet	+30 ppm iron	50 - 100%	50%	85%	45%	75%
Sweetpotato	+70 ppm pro-vitamin A	50 - >100%	0%	≥100%	0%	≥100%
Rice	+12 ppm zinc	50 - 75%	40%	70%	40%	70%
Wheat	+12 ppm zinc	50 - 100%	49%	73%	24%	35%

Source: https://www.harvestplus.org/content/estimated-average-requirements-provided-biofortification selectivity, high performance liquid chromatography (HPLC) is the method of choice to quantify individual carotenoids and their isomers in grains, whereas Near Infrared Reflectance Spectrophotometry (NIRS) is widely used for root and tuber crops (Belalcazar et al. 2017; Sanchez et al. 2014). The XRF and NIRS based measurement methods are substantially less expensive than ICP or HPLC based methods.

Conventional Plant Breeding

Figure 2 displays a conceptual framework for breeding biofortified germplasm and outlines the key activities in developing biofortified germplasm and reflects the impact pathway. The left column contains activities outside of crop development to ensure nutritional impact and farmer and consumer acceptance. The right columns reflect sequentially arranged stages and milestones in crop development, and are superimposed upon a decision-tree that allows monitoring progress and making strategic decisions if goals and targets cannot be achieved.

Crop improvement activities for biofortification focused initially on exploring the available genetic diversity for iron, zinc, and provitamin A carotenoids (yellow boxes) in the germplasm in ongoing breeding programs, and assessing diversity in the largely unimproved germplasm in core collections in gene banks, including wild relative species and unimproved stocks such as landraces. In parallel, in field evaluation or during subsequent screening, agronomic and end-use features are characterized, as varieties must provide good crop yields as well as marketing options to farmers. Objectives when exploring the available genetic diversity are to (1) identify micronutrient dense parental genotypes to be used in crosses, pre-breeding/parent building, development of molecular-markers associated with higher trait levels, genetic and crop physiological studies, and (2) identify existing varieties, pre-varieties in the breeding and release pipeline, or germplasm in final product development stage for "fast-tracking." Identifying already developed varieties or hybrids and commercializing these genotypes that combine the target micronutrient density with the required agronomic and end-use traits enables "fast tracking" in which they are delivered immediately. If suitable variation for micronutrients is present only in unadapted sources in the strategic gene pool, pre-breeding is necessary prior to using the trait in final product development, as the trait needs to be combined with commercially used genetic backgrounds.

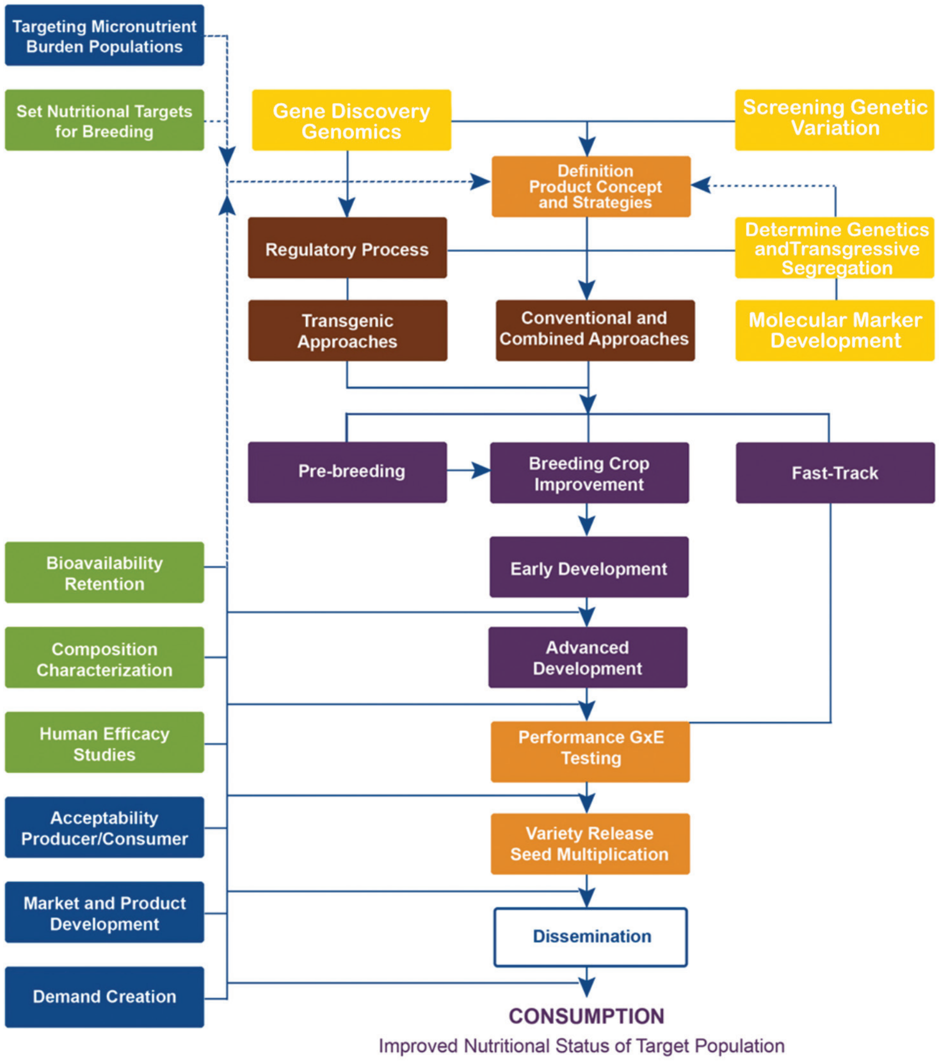


Figure 2. Crop Development Framework. Source: HarvestPlus

The difficulty in using unadapted sources in breeding may involve large genetic distances, the need for elimination of other unfavorable traits that initially come along with selection for the target trait, which adds to product development time and costs. If adequate variation is present in the adapted gene pool, the trait donors can be used directly to develop competitive varieties (purple boxes). Some breeding programs simultaneously conduct pre-breeding and product enhancement activities to develop germplasm combining high levels of one or more micronutrients. Once micronutrient density is available in adapted, high yielding background for target agro-ecologies and production conditions, the need for pre-breeding efforts decreases.

The next breeding steps involve: developing and testing micronutrient-dense germplasm, continuing to conduct genetic studies, further developing and using molecular markers for micronutrients, identifying loci for bioavailability and developing associated markers, genomic selection, and speed breeding with rapid generation advance to facilitate breeding progress (Gebremeskel et al. 2018; Guo et al. 2020; Mageto et al. 2020a, b; Menkir et al. 2018; Owens et al. 2014, 2019; Prasanna et al. 2020). In general, yield, agronomic and end-use characteristics are first considered in selection, as these traits trigger adoption by farmers and cannot be compromised. Breeding efforts for cross-pollinated crops focus on developing hybrids, but also can involve synthetics and open-pollinated varieties on a smaller scale during a transitional period, until formal and informal hybrid seed systems are established, become reliable, and hybrid seed costs are low enough to be accessible to small holder farmers. GxE—the influence of the long term climatic conditions and the more seasonal environmental factors on micronutrient expression—is then assessed at experiment stations and in farmers' fields in the target countries (orange boxes), along with agronomic experiments to develop crop management recommendations for maximizing trait expression and yield. These experiments focus on sustainable agronomic practices such a minimum tillage, direct seeding, residue retention with reduced water use to enhance soil fertility and reduce the environmental footprint. The most promising varieties from multi-location testing over multiple seasons by national research partners, are then submitted to national government agencies for testing for agronomic performance and release, a process which typically takes two years, sometimes more. A key element in on-farm testing is Participatory Variety Selection (PVS). Farmers test the agronomic and end-use attributes of candidate varieties in PVS under their relevant situation and are genuine partners in breeding and guiding variety release recommendations.

International nurseries/global testing

HarvestPlus has used two strategies to shorten time to market for biofortified crops: (1) identifying already adapted varieties with significant micronutrient content for immediate release and/or dissemination as fast track varieties, while varieties with target micronutrient content are still under development, and, (2) deploying multi-location Regional Trials with elite materials including released varieties across a wide range of countries and sites each growing season (Andersson et al. 2017). These regional or transcontinental nurseries serve in germplasm dissemination as well as a testing tool. Agronomic and micronutrient data from multiple sites per country allows high precision identification of fast-track candidates or inbred lines for breeding, it generates data on yield and micronutrient stability, and permits identifying the adaptive pattern of the germplasm to the different agro-ecological zones. By including end-use requirements, the germplasm can be grouped and targeted when tested in new countries. Further, by substituting assessment with temporal environmental variation with spatial environmental variation in large scale regional GxE testing, testing steps can be eliminated and time-to-market shortened by 1–2 years. Release in other countries is further accelerated by regional agreements, which harmonize seed regulations of member countries, and allow faster release if a variety has been released in similar agro-ecologies in one of the regional countries.

Strategic priorities

The key longer term priority is mainstreaming biofortification. Mainstreaming refers to incorporating micronutrient density as a core trait in essentially all breeding programs developing varieties. Consequently all offspring, all future varieties will be biofortified and the breeding effort required will be reduced to maintenance breeding. Recent progress in developing molecular markers associated with higher levels of micronutrients will help facilitate mainstreaming (Babu et al. 2013; Swamy et al. 2016).

Medium term objectives aim at improving bioavailability of iron and zinc, for example by decreasing the anti-nutrient phytate, or increasing phytase, the enzyme that degrades phytate. For Vitamin A crops, breeding pursues increasing the retention and stability of provitamin A carotenoids.

Strategic priorities in breeding shorter term center on strengthening the pipeline of biofortified varieties by developing next waves of competitive, climate smart crops with target nutrient levels and broader adaptation across agro-ecological zones, production conditions and end-uses. Breeders can develop biofortified crops by directly breeding for increased micronutrient concentration, and/or higher bioavailability.

Releases of Biofortified Crops

Cumulatively, more than 340 biofortified varieties of 12 crops have been released in more than 40 countries, which includes orange sweetpotato varieties developed by CIP. Candidate biofortified varieties across 12 crops are being evaluated for release in an additional 20 countries. Figure 3 depicts where biofortified varieties have been tested and are released to date.

Chapter 4.

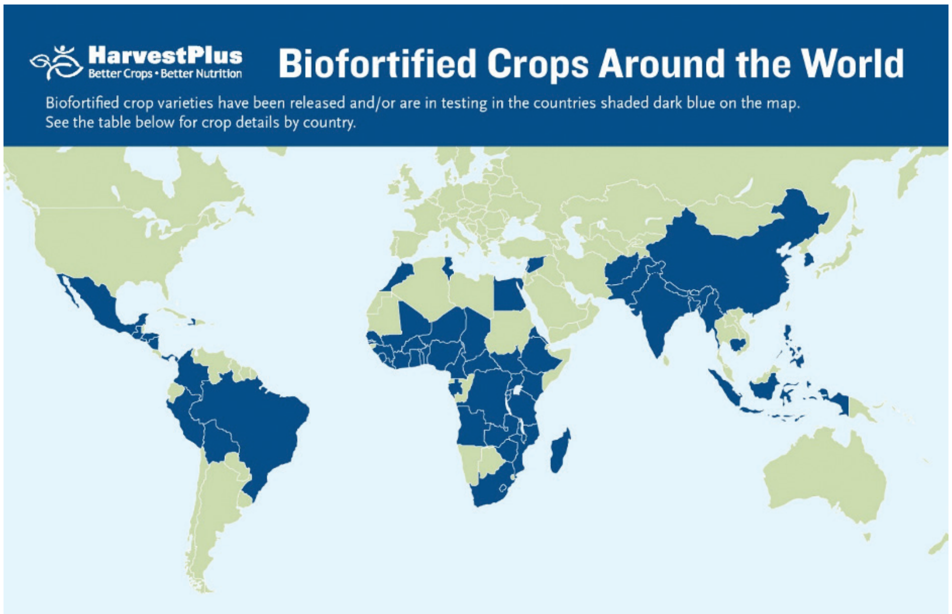
Delivery and Demand Creation Strategies Tested and Lessons Learned

Since 2010, the International Potato Center (CIP) and HarvestPlus have engaged in major efforts to collaborate with public sector, private sector, and NGO partners to bring biofortified planting materials to farming households, and biofortified foods to consumers. This required linking farmers and consumers into the biofortified seed to food value chain in very diverse environments and institutional settings in Africa, Asia, and Latin America.

By the end of 2019, HarvestPlus-led delivery efforts for iron beans and pearl millet, vitamin A cassava and maize, and orange-fleshed sweetpotato (OFSP), and zinc rice and wheat were benefiting an estimated 8.5 million farming households, while 6.8 million farming households were reached with OFSP vines through partners in the Sweetpotato Profit and Health Initiative (SPHI) co-led by CIP and the Forum for Agricultural Research in Africa. Additional organizations are now working to integrate biofortified crops in their programs, to contribute to the transformation of food systems to deliver healthy foods for all.

Progress on measuring adoption is however slower, as such studies should reflect cultivation practices at least a couple of years after the dissemination effort and require significant funds to conduct. For commercialized seed systems, monitoring sales can be an effective proxy. Studies documenting adoption are available for iron bean adoption in Rwanda (Vaiknoras et al. 2019), vitamin A maize in Zambia (Diressie et al. 2016), and OSFP in Uganda and Mozambique (de Brauw et al. 2018). Many more are expected during the coming decade.

This chapter provides a summary of the tested delivery strategies and learnings from delivery of biofortified planting material to increase varietal adoption and demand of biofortified foods. Chapter 5 covers essential concurrent activities to create the enabling environment to support delivery at scale, designed to reach specific vulnerable groups or distinct sub-regions and agro-ecologies.



Biofortified Crop Varieties Released (R) or in Testing (T) by Country

HIB = Iron Beans
IPM = Iron Pearl Millet

ZIM = Zinc Maize
ZIR = Zinc Rice
ZIW = Zinc Wheat

ABP = Vit. A Banana/Plantain
VAC = Vit. A Cassava
VAM = Vit. A Maize
OSP = Vit. A Orange Sweet Potato

IZC = Iron/Zinc Cowpea
IZP = Iron/Zinc Irish Potato
IZL = Iron/Zinc Lentils
ZIS = Zinc/Iron Sorghum

Source: HarvestPlus, International Potato Center (2019)

Africa	HIB	IPM	ZIM	ZIR	ZIW	ABP	VAC	VAM	OSP	IZC	IZP	IZL	ZIS
Angola								T	R				
Benin Rep		T					T	T					
Burkina Faso		T						T	R				
Burundi	R					R			R				
Cameroon						T	R	R					
Central African Rep							T						
Chad							T						
Côte d'Ivoire						T	T		R				
DR Congo	R					R	R	R					
Egypt					T			T					
Eritrea		T							T				
Ethiopia			T		T		T	T	R		T	R	
Gabon							T						
Gambia		T					T						
Ghana		T					R	R	R				
Guinea						T	T						
Kenya	T	T					T	T	R		T		
Liberia							T	T					
Madagascar		T		T					R				
Malawi	T	T					T	R	R				
Mali		T						R	T				T
Morocco									T			T	
Mozambique							T	T	R				
Niger		R					T	T	T				
Nigeria		T	T			T	R	R	R	T			T
Rwanda	R					T		R	R		T		
Senegal		T		T			T	T	T				
Sierra Leone							R	T					
South Africa								T	R				
South Sudan		T						T	T				T
Swaziland							T						
Tanzania	R	T				T	T	R	R				
Togo		T						T					
Tunisia		T											
Uganda	R	T				T	T	T	R		T		T
Zambia		T			T		T	R	R				
Zimbabwe	R	T			T			R	T				

Asia	HIB	IPM	ZIM	ZIR	ZIW	ABP	VAC	VAM	OSP	IZC	IZP	IZL	ZIS
Afghanistan					T								
Bangladesh				R	R				R			R	
Bhutan					T						T		
Cambodia				T									
China				T	T			T	R		T		
East Timor									R				
India		R		R	R			T	R	R	T	R	R
Indonesia				R					R				
Lebanon												T	
Myanmar				T									
Nepal					T			T			T	R	
Pakistan					R			T				T	
Philippines					T								
South Korea									R				
Syria												R	

LatAm/Caribbean	HIB	IPM	ZIM	ZIR	ZIW	ABP	VAC	VAM	OSP	IZC	IZP	IZL	ZIS
Bolivia	R		R		R						T		
Brazil	R			T	T		R	R	R	R			
Colombia	R		R	T			T	T	R				
El Salvador	R		T	R									
Guatemala	R		R	T			T		R				
Haiti	T		T	T				T	T				
Honduras	R		R						T				
Mexico			T		R			T					
Nicaragua	R		R	T					R				
Panama	R			T	T		T	T	R				
Peru									R		T		

Figure 3. Biofortified Crop Variety Release and in-Testing

Delivery Strategies for Vegetatively-Propagated Vitamin A OFSP and Vitamin A Cassava (VAC)

Vegetatively propagated crops (VPC)—those for which farmers plant stems, tubers, or cuttings/vines rather than seeds—typically have seed systems characterized by farmer multipliers varying in size of operation and degree of commercialization. Planting materials are perishable and bulky, making them expensive to transport over long distances and require planting within a few days of harvesting. The lack of commercial private sector participation creates both a challenge and an opportunity for producing planting materials of biofortified crops like vitamin A OFSP distributed as vines and vitamin A (yellow) cassava (VAC) distributed as stem cuttings. Public sector support, for pre-basic material (early generation seed), often plays a critical role. Farmers share planting material of VPC within their social networks. Farmer-to-farmer diffusion is therefore a key driver for widespread diffusion of all VPCs.

Research efforts on VPC "seed" systems have typically focused on how to ensure disease-free starter planting material of adapted biofortified varieties to enable multipliers retain sufficient quality and produce enough vines/stems over time. In addition, extension has focused on training farmers on conserving and maintaining quality planting material. In drought-prone areas, the root-based multiplication technique for sweetpotato known as Triple S (Storage in Sand and Sprouting) has enabled smallholder farmers to preserve sweetpotato planting material during months-long dry seasons.

For OFSP, there have been two major delivery strategies, the first based on establishing networks of trained Community Based Vine Multipliers (CBVM); the second mass dissemination efforts usually associated with emergency response where public sector multiplication and a few large-scale multipliers are the core sources of planting material (Low et al. 2017). For both, CIP and national program scientists develop adapted OFSP varieties, engaging farmers in their evaluation. The most promising cultivars are released by the country.

Uganda, where HarvestPlus has coordinated the overall OFSP scaling up effort, is an excellent example of the first delivery strategy. A contracted private sector tissue culture laboratory provides pre-basic or foundation seed. The resulting disease-free cuttings are provided to contracted and trained CBVMs for further multiplication. Those in high virus pressure areas are provided with mini-screenhouses to maintain pre-basic seed protected from vector insects.

Vine distribution partners are contracted to train farmers and to distribute seed loan vines to farmers who agree to pay the loan by giving a prescribed quantity of vines to three other farmers, or by paying it back to distribution partners for onward distribution to new farmers. The use of community and radio dramas, lead mother groups as entry points and field days as learning platforms, are key to create awareness and stimulate demand for vines and roots. To create demand for OFSP roots, farmers are linked to processors, fresh produce markets and institutional buyers. In 2019, 15% of sweetpotato farmers were growing OFSP in Uganda, compared to 1.2% in 2010 (Walker and Alwang, 2015).

In Mozambique, as a response to widespread drought or floods pre-basic seed and large-scale multiplication fields are established at government stations while a few commercial farmers provide large quantities of vines for mass distribution efforts. Local leaders, extension personnel, and radio messaging inform communities in time of when and where to get vines to enable advance land preparation. A one-shot nutrition awareness campaign is conducted. As of 2015, one third of all sweetpotato grown in Mozambique was orange-fleshed, compared to 14% in 2005 (Dept. de Estatística 2005).

Nigeria has so far, the largest VAC promotion program. Following the release of the VAC varieties, smallholder and large-scale commercial VAC stem multipliers are contracted to multiply quality declared stems while stem distribution partners are contracted to raise awareness, distribute promotional stem packs, and train extension staff to implement step-down cascade trainings to farmers. Farmers "pay" for the planting material received by giving stems to three other farmers in their network, in the next season—enabling resource-poor farmers, especially women, to access VAC stems. In subsequent years, increased stem production is matched by crowding in public, private and NGO partners to significantly increase the demand for stems and enable VAC stem production to become a business. VAC growers are linked to processors of healthier VAC-based food products. In 2019, 8% of cassava area was planted with VAC, starting from none in 2013.

Delivery Strategies for Self-Pollinating Iron Beans, Zinc Rice, and Zinc Wheat

Delivery models for self-pollinated biofortified crops were tested for zinc wheat (ZW) in India and Pakistan, zinc rice (ZR) in Bangladesh and India, and iron beans (IB) in Democratic Republic of the Congo (DRC), Kenya, Rwanda, and Zimbabwe. While farmers do need to periodically replace their seed to maintain its desirable agronomic traits, the relatively small annual market for seed typically limits private sector investment in producing seed for self-pollinated crops.

In many countries, public sector commercial seed companies and Community Based Seed Multipliers (CBSM) multiply and distribute seed while farm-saved-seed and farmer-to-farmer seed contribute significantly to varietal diffusion. To promote adoption these crops following their release, public sector commercial seed companies supported and CBSM are contracted, to multiply and distribute seed. Both are supported to access foundation seed to meet agreed seed quality and production targets that are set each season.

Several countries permit the use of quality declared or truthfully labeled seed categories, enabling rapid seed multiplication. Seed distribution partners are contracted and then trained to raise awareness, distribute promotional seed packs, and train extension staff who will do a step-down cascade training to farmers. The aim is to distribute seed loan packs to farmers who will pay back in the following season by giving a prescribed quantity of their farm saved seed to three other farmers, thus reaching low income and vulnerable households. The pay forward strategy is cost-effective and efficient for targeting low resource households for IB, ZR, and ZW. This strategy, combined with the use of farm-saved-seed and high voluntary farmer-to-farmer seed sharing resulted in high adoption rates for IB in Rwanda where 20% of the total bean production was iron beans and with 15% of the population consuming them only after five years of active promotion. Likewise, in 2019, 24% of wheat growers in India's Bihar state were growing ZW after four years of active promotion.

We also found that well-designed farmer demonstration plot programs combined with farmer field days are key pillars for rapid popularization of IB, ZW and ZR. Price guarantees, price subsidies and, providing technical and financial support for promotional work proved to be effective de-risking strategies. Farmers grow not only those varieties they prefer to consume themselves, but also those they can sell easily. Therefore, increasing consumer awareness, creating products and markets, and developing grain aggregation capacity are all essential for stimulating demand for biofortified crops.

Delivery Strategies for Hybrid Vitamin A Maize and Iron Pearl Millet

There are hybrid and open pollinated varieties (OPV) of both hybrid vitamin A maize (VAM) and iron pearl millet (IPM). For hybrid varieties, seed must be replaced each year to maintain the yield and other agronomic traits, while for OPV farmers can use farm-saved-seed for up to four years without significant yield drops. While seed for hybrid varieties offer the most potential for commercialization, OPVs do not. VAM hybrid varieties are promoted in Malawi, Nigeria, Tanzania, Zambia, and Zimbabwe; while VAM OPV varieties are promoted in DRC, and Nigeria, and both hybrid and OPV varieties of IPM in India. NARS and some CGIAR partners are supported to provide foundation seed to private and public sector commercial seed companies licensed to multiply VAM and IPM seed.

The existing seed systems for these crops are well-developed, commercial, and robust. Biofortified varieties can be easily integrated into the existing seed production and distribution infrastructure. To increase speed of private sector uptake, initial de-risking is often required, particularly support for demand creation for farmers and consumers. For both hybrids and OPVs a robust program of demonstration plots, training of extension and retail staff, farmer field days and distribution of promotional material containing agronomic and nutritional benefits messages are all essential. Integrating VAM and IPM varieties in government input distribution programs for Zambia and India was critical for the rapid adoption and production of VAM and IPM varieties, respectively. Strengthening the capacity of VAM aggregators and linking them to private sector processors was essential for stimulating demand for VAM grain while supporting the development of grain standards will be crucial for driving widespread adoption of VAM in Nigeria, Zambia, and Zimbabwe. The penetration of VAM hybrids is greatly limited by the need to replace seed every season, and may exclude resource poor farming households, especially female headed ones, mandatory pay forward and payback systems, and voluntary farmer-to-farmer sharing of seed for OPVs rapidly increased access to VAM by resource poor farmers in DRC and Nigeria. By end of 2019 (after three seasons of promotion), 4% of maize area was planted with VAM in Nigeria.

We know that market opportunities for specific varieties accelerate adoption. A study of farmer-to-farmer diffusion of OFSP concluded that having a critical mass of farmers cultivating the crop increased the likelihood of sustained cultivation. To maximize diffusion, focus should be on dissemination of OFSP vines to farmers who know many other farmers in areas where there will be significant returns to OFSP cultivation (McNiven and Gilligan, 2012).

Consumer Demand for Biofortified foods

Demand for biofortified foods from both rural consumers—who may or may not also be the producers of biofortified crops—and urban consumers, is what is expected to drive the production and consumption of biofortified crops. In order to understand consumer demand for biofortified foods, various consumer acceptance studies were conducted by testing consumer valuation (captured as willingness to pay [WTP] through revealed choice experiments, experimental auctions and auction like mechanisms [such as the Becker-DeGroote-Marschak mechanism] or in terms of their sensory evaluation [captured through hedonic rating with 5 or 7 point Likert scales] or both) of biofortified vs non-biofortified types of the most commonly consumed preparation of that staple food in a country. Overall, consumers' acceptance of the biofortified varieties has been very promising, as summarized in review papers such as Birol and colleagues (2015) and Oparinde and Birol (2019). According to these reviews, biofortified crops are liked by target consumers. In some cases, consumers preferred biofortified food to non-biofortified food even in the absence of information about the nutritional benefits of biofortified foods, though information and awareness campaigns often have an important role to play. This finding is important for proving the acceptability of both vitamin A biofortified crops—which change color and some other organoleptic characteristics due to their beta-carotene content, as well as for mineral-biofortified crops, which don't have any visible changes, and hence may not be considered as more nutritious than their conventional counterparts.

For the vitamin A enriched crops, the bright color has been used as an effective marketing tool. The association between the color (yellow or orange) and vitamin A content is easily established and decorated market stalls, clothing, vehicles, and other promotional items can contribute significantly to increasing awareness and building demand. Broader links to include other nutritious vitamin A foods can also be easily made.

Vitamin A Biofortified Crops

Orange-fleshed sweetpotato (OFSP) – Investing in breeding for higher dry matter varieties of OFSP in Africa was critical for gaining adult consumer acceptance of orange-fleshed types. The color itself was never a barrier as both children and adults always found it attractive (Low and Thiele 2020). Sensory evaluation studies conducted in Uganda (Chowdhury et al. 2011), Mozambique (Stevens and Winter-Nelson 2008; Laurie and Van Heerden 2011), South Africa (Pillay et al. 2011) and Malawi (Hummel et al. 2018) showed that consumers liked the sensory attributes of OFSP, as well as those of various processed products (e.g., bread, chapatis, chips, doughnuts, and juice) made with OFSP. WTP studies conducted in rural areas of Uganda revealed that when nutrition information on the benefits of OFSP was provided, consumers valued orange varieties more than white ones (Chowdhury et al. 2011). Another WTP study in Mozambique found that consumers valued OFSP and that value was enhanced by information on nutritional benefits (Naico and Lusk 2010). Clearly, information campaigns help drive demand for OFSP.

Vitamin (orange) A maize (VAM) - In rural Zambia, consumers valued more highly *nshima* made with VAM compared with to *nshima* from white and yellow maize varieties, even in the absence of nutrition information. Providing nutrition information, however, also translated into consumers valuing VAM more (i.e., willing to pay more for VAM seed), signaling the higher levels of benefits accrued from this nutritious food (Meenakshi et al. 2012). Two media channels (simulated radio messaging and community leaders) were used to convey the nutrition message. The study found consumers valued VAM similarly regardless of the media source, implying that radio messaging, which is significantly less costly than face-to-face message delivery, can be used to convey nutrition information. Another study, conducted in rural Ghana, found that consumers valued *kenkey* made with VAM less than *kenkey* made with either white or yellow maize, but the provision of nutrition information reversed this preference. Thus, an information campaign will be key to driving consumer acceptance of VAM in this country (Banerji et al. 2018).

Vitamin A (yellow) Cassava (VAC) - A study was conducted in Oyo and Imo states of Nigeria to understand consumer preference for VAC *gari*[7] compared to local *gari*. In Oyo, the local *gari* evaluated was made with white cassava, and in Imo it was yellow (white cassava mixed with red palm oil), in accordance with regional preferences. In Oyo, consumers preferred *gari* made with light yellow colored VAC even in the absence of nutrition information. Once consumers received information about the nutritional benefits of VAC varieties, light yellow-colored VAC remained the most popular, but *gari* made with deeper-yellow colored VAC was preferred over the local variety. In Imo, on the other hand, in the absence of nutrition information, local *gari* was preferred to the *gari* made with either light- or deeper-yellow colored VAC varieties; however, once consumers were informed about the nutritional benefits of VAC, *gari* made with the deeper-yellow colored vitamin A cassava was preferred—another example of the importance of information campaigns in areas where biofortified crops are introduced (Oparinde et al. 2016a). Another study on vitamin A cassava acceptability in Nigeria, compared traditional West African foods prepared with biofortified, fortified, or conventional products, and found that consumers preferred biofortified products, associating the yellow color of vitamin A cassava with improved eyesight and enhanced health (Bechoff et al. 2018). A consumer acceptance study conducted in Kenya found that both the caregivers (18- to 45-year-olds) and children (7- to 12-year-olds) preferred yellow VAC over white cassava varieties, because of the former's soft texture, sweet taste, and attractive color (Talsma et al. 2013).

Iron Biofortified Crops

Iron pearl millet (IPM) – In rural Maharashtra, India, an evaluation of *bakhri*—a form of flat bread—made with IPM compared to market-purchased pearl millet revealed that even in the absence of information about the nutritional benefits of iron pearl millet, consumers liked the sensory attributes of the grain and *bakhri* of the iron pearl millet variety as much as (if not more than) those of the conventional variety. Nutrition information, however, significantly increased consumer preferences for IPM *bakhri* (Banerji et al. 2016)

Iron Beans (IB) – Consumer acceptance studies conducted in rural Rwanda showed that even in the absence of nutrition information, consumers in the Northern Province liked the sensory attributes of a red IB variety more than a white IB or local bean variety. Nutrition information had a positive effect on the premium consumers in urban wholesale and retail markets were willing to pay for IB: when provided, both IB varieties were preferred to the local variety (Oparinde et al. 2016b). When compared across regions, consumers in the rural Western Province and urban wholesale market also had similar preferences for one of the IB varieties tested, suggesting potential for linking demand and supply (Oparinde et al. 2017). Another analysis of multiple sensory attributes revealed several opportunities for marketing of IB in both rural and urban markets (Murekezi et al. 2017). Similar studies conducted in the LAC region, (in Colombia [Beintema et al. 2018] and Guatemala [Perez et al. 2018]), also revealed that consumers like IB at least as much as their most popular local bean varieties.

Making a Difference to Segments Most at Risk of Deficiency

Getting a household to grow a new biofortified variety is just part of the equation if the goal is to change the nutritional status of the most vulnerable household members, particularly children under two years of age and their mothers. Delivery systems that include community level nutrition education programs to improve young child feeding practices using OFSP as a key intervention have clearly demonstrated improved vitamin A intakes in young children and their mothers and vitamin A status in young children (Hotz et al. 2012; Low et al. 2007). Another model linking improved nutritional counseling and access to OFSP planting material to ante-natal care services for pregnant women tackled the critical period of improving nutrition knowledge and dietary practice among

[7] *Gari* is a flour made from grated fresh cassava with the excess liquid dried out.

pregnant women and lactating mothers (Cole et al. 2016; Girard et al. 2017). Clearly, biofortified crops can be included in integrated nutrition-sensitive programs to address the needs of those groups most at risk of micronutrient deficiencies in a holistic manner that includes influencing the social environment to support required behavioral change for impact (Ruel et al. 2013).

Conclusion

History has shown that scale up of modern varieties of crops developed through public breeding programs require substantial public investments to jump-start the process of farmer and consumer adoption. This is not exclusive to biofortified varieties, but to all modern varieties. Once the process has been initiated, private market actors can sustain and build on this momentum based on increased crop productivity and growing market demand, though in some cases public and NGO / humanitarian sectors might need to remain involved to serve low income and other vulnerable populations that cannot access/afford improved seed, and especially for crops which don't lend themselves to private seed sector investments due to perceived or actual lack of cost-recovery because on-farm seed retention and farmer-to-farmer diffusion predominate.

The public sector has recognized the public health value of putting more iron, zinc, and vitamin A in the edible portions of crops through crop breeding and initiated a process in which the private sector did not initially participate. Countries with stronger public sector agricultural extension services have been able to scale faster than those with weaker systems. These examples of scale up of crops in specific countries have shown how the public and private sectors should and can work together to improve the nutritional quality of staple foods in developing countries. Future generations will continue to reap the rewards of these initial investments.

Chapter 5.

Catalyzing the Scale Up of Biofortification

The previous chapter has provided details of actions to initiate supply and demand requirements for rapid scale up of specific crops in national settings, based on market-led forces. It is essential strategically to reinforce these efforts through communications, advocacy, policy, and related measures to ensure that resources and public cooperation are in place to give further momentum to the market-driven supply-demand efforts. The primary areas of activities are

- Mainstreaming biofortification breeding in public and private agricultural research,
- Inclusion of biofortification in national and regional policies, programs and regulations,
- Inclusion of biofortification in international financial institutions' loan portfolios
- Facilitating the "biofortification" of seed to food value chains for staple crops,
- Inclusion of biofortification in humanitarian programs.

Each one of these mechanisms are explained in greater detail below, and the chapter is concluded with remarks on the role of biofortification in improving food systems, especially under the challenging conditions set forth by the global pandemic and climate crisis.

Mainstreaming biofortification breeding in public and private agricultural research

A central tenet of a successful, long-term biofortification strategy is that all future varieties developed by CGIAR Centers, NARS, and private seed companies be biofortified. By adding micronutrient density to best performing varieties coming out of public and private breeding programs, currently grown varieties would eventually be replaced by higher-yielding biofortified varieties—thus "mainstreaming" these characteristics into all varieties. Although mainstreaming will take time as all major breeding lines will have to be biofortified, this international and national public research strategy for improving the productivity and quality (nutrient content) of key crops grown and consumed by rural poor is highly cost-effective.

From a consumer standpoint, mainstreaming is easiest to accomplish for iron- and zinc-biofortified crops, as increased micronutrient levels in the crops are invisible to consumers in seeds and grains. Uptake does not depend on changing consumer behavior, and is often automatic and inevitable, relying on the profit- incentive of farmers. Success has been seen in production and consumption of zinc-biofortified rice and wheat, and iron-biofortified pearl millet in South Asia and iron-biofortified beans in East Africa. This strategy does not work as easily for vitamin A-biofortified crops in countries where white color cassava, sweetpotato, or maize dominate; increasing the density of vitamin A changes the color of these crops to yellow in the case of cassava and orange in the case of maize and sweetpotato and can affect taste as well (albeit positively as evident from Operinde and Birol 2019). Demand must be generated for these varieties by raising consumer awareness on nutritional and agronomic benefits of biofortified varieties. Such demand creation efforts can be easily linked to broader nutrition education strategies. In most settings color and taste are not barriers to consumer demand once household members taste the vitamin A varieties and WTP for the new variety can increase once consumers understand the reason for the color change, as explained in previous section.

Investing in "targeted" breeding programs at CGIAR Centers and NARS was key to developing competitive biofortified varieties and to proving the concept of high-yield and other agronomic traits farmers and consumers like could be combined with mineral and vitamin density, but it also built crop development pipelines. Mainstreaming quality traits requires using high-throughput testing of each clone using specialized equipment, which does increase the annual budgets of breeding programs. However, in the case of the visible vitamin A trait, in early stages clones can be separated inexpensively through visual evaluation. The time has come to start transitioning out of targeted breeding for biofortification, and to move to mainstreaming of nutritional traits in broader germplasm of major staple crops. Biofortification mainstreaming would help address micronutrient needs of billions of people whose diets are based on these staples, both sustainably and cost-effectively. For example, breeders in14 national sweetpotato breeding programs in Africa have signed a pledge that 50% of the varieties they will submit to release committees will be orange-fleshed. The CGIAR has a critical role in providing biofortified parental material to NARS and/or access to high-throughput testing equipment to accelerate their ability to develop locally adapted biofortified varieties and the overall efficiency of biofortification efforts.

Inclusion of Biofortification in National and Regional Policies, Regulations and Programs

In order to ensure sustainability of biofortification it is crucial to ensure biofortification is included in national policies, strategies, plans and programs, and then specific budgets/funding is allocated to implement these policies/strategies and plans.

Twenty four countries have now included biofortification in their national agricultural and/or nutrition agendas, policies, plans and programs (including Bangladesh, Burkina Faso, Burundi, DRC, Colombia, El Salvador, Ethiopia, Ghana, Guatemala, Honduras, India, Kenya, Malawi, Mozambique, Nicaragua, Nigeria, Pakistan, Panama, Rwanda, Senegal, Tanzania, Uganda, Zambia, and Zimbabwe). Several of these countries have included biofortified crops/foods in their programs, for example vitamin A maize varieties are included in Zambia's Farmer Input Subsidy Program (FISP) (Mwale 2020) which provides subsidies for maize seed, among other inputs; orange-fleshed sweetpotato in the President's Planting for Food and Jobs Initiative in Ghana; in Nigeria extension services in a total of 34 states are now delivering biofortified planting material, as a result of initial biofortification program success in the first four states. Most recently the Government of Bihar state in India has committed to scaling up production and consumption of biofortified crops (HarvestPlus 2020a).

At the regional level there has been significant progress with the inclusion of biofortification in African Union (AU)'s Comprehensive Africa Agriculture Development Program (CAADP). The AU's Executive Council, as well as AU members' ministers of agriculture, have endorsed recommendations on biofortification (HarvestPlus 2019), and heads of state are expected to follow suit at their next Summit. The African Union's Business Plan to Implement the CAADP-Malabo Declaration (2017-2021) (CAADP 2017) refers to biofortification as a strategic thrust under the Malabo result area "Ending Hunger in Africa by 2025". African Union Development Agency New Partnership for Africa's Development (AUDA-NEPAD) 2019-2025 Nutrition and Food Systems Implementation Plan (NEPAD 2019) highlights biofortification among high impact actions to address hidden hunger, in one of the flagships, core intervention or cross-cutting areas. Most recently, the European Commission's Guidance note on fortification (July 2020) includes an endorsement of supporting biofortification, to ensure access to nutritious and safe food for all, and to create jobs, promote entrepreneurship, and inclusive economic growth.

At the international level, several UN and Rome-based agencies have integrated biofortification in their recommendations and programs, such as the inclusion of biofortification in Unicef's the State of the World's Children 2019 Report (UNICEF 2019), and in World Food Programme's local and regional food procurement policy (WFP 2019). To strengthen the global enabling environment for integration of biofortification in food systems as a means for improving food and nutrition security, for example, HarvestPlus will continue to engage with and provide evidence to the World Health Organization (WHO) Guidelines Review Committee to facilitate their issuance of evidence-based guidance to UN member states' health and agriculture ministries and provide input to the UN Committee on World Food Security (CFS) Voluntary Guidelines on Food Systems and Nutrition.

With regards to standards, national level examples include the minimum iron and zinc breeding targets set for pearl millet in India and iron bean seed and grain standards in Rwanda (HarvestPlus 2019b). At the global level considerable progress toward a global definition for biofortification was achieved through the FAO/WHO *Codex Alimentarius*. Current work is focusing on working with the International Standards Organization and creating international nutrient standards for biofortified grains through the Publicly Available Standards process. There are also concurrent efforts to include production and consumption of biofortification in nationally representative surveys (such as DHS, HCES and other agricultural production and food consumption surveys implemented by governments and other agencies) to monitor the reach of biofortified crops and foods.

Inclusion of Biofortification in International Financial Institutions' Loan Portfolios

Inclusion of biofortification intervention in policies, programs, and loan portfolios of international financial institutions (IFIs), can be considered as the opposite side of the coin to inclusion of biofortification in national policies and programs. Several IFIs – such as the World Bank and the International Fund for Agricultural Development (IFAD) now have nutrition targets for their loans to the agriculture sector, and biofortification is considered as a cost-effective and shovel ready technology that requires minimum behavior change and infrastructure investment to implement. Biofortification is included in IFAD's Nutrition Sensitive Value Chains guidelines (IFAD 2018), and in African Development Bank's Multi-Sectoral Nutrition Action Plan (AHHD 2018), as well as in several World Bank documents on how to deliver nutrition sensitive agriculture (e.g., Dizon, Josephson, and Raju 2019) and on biofortification being a prime example of nutrition smart agriculture investments (Arias and Htenas 2019), and in an increasing number of World Bank loans (see examples in Malawi [HarvestPlus 2020c] and Uganda [Shekar et al. 2016]).

Work will continue with these IFIs and

several others (such as Asian Development Bank and Islamic Development Bank) as well as with the national governments, to provide them with information, guidance and technical assistance on how to decide whether or not to integrate biofortification in their policies, programs and loans (e.g., providing information on not only nutritional efficacy, but also on ex ante cost-benefit/ cost-effectiveness analysis and on return on investment of including biofortification in specific policy and regulatory changes and specific public programs [e.g., subsidy or safety net programs]); on developing biofortification programs (e.g., targeting for impact, development and implementation of delivery models), and on how to track the process, evaluate the impact, conduct cost-benefit analyses of biofortification investments.

Facilitating the "Biofortification" of Seed to Food Value Chains for Staple Crops

A value chain approach is essential for sustainable mainstreaming of biofortification in seed and food systems. Stakeholders along the seed to grain to food value chains for each biofortifiable staple crop should be catalyzed and capacitated to supply and demand biofortified products, as follows:

For crop research and development, as CGIAR Centers mainstream biofortification in their breeding programs, they will also continue to assist NARS and the private seed companies —mostly small and medium-scale – in capacity development for biofortification breeding and pre-basic seed production; use of diagnostic tools; identification of best germplasm in breeding; selection of best breeding environments, and micronutrient bioavailability and retention. HarvestPlus fostered the development of now independently funded biofortification programs in Brazil (e.g., Nutti et al. 2009), China (Pray and Huang 2007), and India (Yadava et al. 2018). The hope is that these three programs will continue to develop and assist others NARS in the global south.

For seed multiplication, as mentioned in the previous chapter, partnerships with private seed companies are particularly important for hybrid crops such as maize and pearl millet, and in some cases with wheat and rice. For crops which don't lend themselves to investments by the private sector, as also explained in previous chapter, public sector multipliers as well as NGOs, women's groups, farmer organizations, and commercially oriented individual farmers should be involved through information campaigns (to engender demand) and access to finance for and trainings on producing high quality biofortified planting materials.

Distribution to and demand creation for farmers. Working with NARS in particular investment in capacity development of young researchers ensures that biofortified varieties are nationally adapted and owned, and integration of biofortification in national breeding programs are sustainable. Emphasis on working with NARS and in particular with men and women farmers in the country for co-development of biofortified varieties facilitates their maximum and sustained adoption. Capacity strengthening materials co-developed with NARS and farmers as well as other key stakeholders (e.g., influential actors in the seed to food value chains, [such as traders, processors, and urban consumers], nutrition and health community) focus on general nutrition information, including the value of growing and consuming biofortified crops, improved agronomy, including crop rotation, pest management, how to maximize production, accessing markets, and the use of digital technologies for doing so. The internet provides an effective means to share all the learnings on nutritional messaging, agronomic training material and marketing with relevant partners. Regular outcome monitoring and farmer feedback surveys conducted helps improve further development of varieties and the mechanisms through which they are delivered.

Food processing and value addition. Inclusion of biofortified ingredients in processed foods would not only help food systems deliver more nutrients, but is a "demand pull" strategy to stimulate adoption and production of biofortified crops at the farmer level, as alluded to in the previous chapter. Information on development of healthy foods that use biofortified ingredients; retention of micronutrients during processing/transportation/storage; maintaining identity preservation (i.e., supply chain integrity), and food safety should be generated and shared with food processors. Support for financing and business development may be needed to enable small and medium-scale enterprises to work in biofortification.

At the retailer and consumer end of the value chain, as explained in greater detail in the previous chapter, several consumer acceptance studies conducted to with both rural and urban consumers showed that foods made with biofortified crops are liked as much as – if not more than – the same foods made with non-biofortified crops, and in several cases even in the absence of information on their nutritional benefits. We have much to learn from the private sector marketers about what makes an idea "stick". According to Health and Health (2007), a prospective product campaign should follow six principles: (1) focus on an idea that is simple, yet profound; (2) stimulate curiosity; (3) present concrete actions to follow; (4) be credible (test it yourself!); (5) trigger emotions; and (6) use stories to encourage people to act. Clearly, with vitamin A enhanced foods and vegetable and fruits in general, promoting eating a diversified set of colors (orange, yellow, green, purple) is obvious—again keeping it simple and not getting bogged down in excessive detail. Experience to date, targeted at consumers of different socioeconomic and demographic characteristics, is enabling the building of a knowledge base on what kind of messaging and media is efficacious, cost-effective, scalable and sustainable in engendering consumer demand for nutritious biofortified foods. It will be important to continue to engage with leading global food manufacturers and retailers to engender global awareness and demand for "naturally-nutritious" biofortified products, to act as demand pull mechanisms.

Inclusion of Biofortified crops/foods in Humanitarian Programs

While participation of both public and private sectors is essential in creating sustainable pipelines and markets for biofortified seed and foods, humanitarian organizations remain important in delivering this nutrition intervention to vulnerable households. For example, the existing global partnership between World Vision and HarvestPlus is an example of how a leading development NGO can incorporate biofortified crops into its existing agricultural programs, linking them to health and nutrition programs. While HarvestPlus provides technical assistance, World Vision takes the lead in delivery—integrating biofortified crops in 17 countries (including Afghanistan, Bolivia, Burundi, Ghana, Lesotho, Papua New Guinea, and South Sudan—where HarvestPlus doesn't have country programs). This type of partnership, whereby biofortified crops are integrated into existing agriculture and nutrition projects or included in collaboratively developed new projects, will continue to be important to reach the most vulnerable households, which may also be the most likely to suffer from micronutrient deficiencies. Local NGOs, such as Programme Against Malnutrition (Zambia), Volunteer Efforts for Development Concerns (Uganda), and international charities, like Caritas and Self-Help Africa, have also been essential partners in reaching vulnerable households with biofortified crops. Regional NGOs, such have Farm Concern International, have focused on organizing farmers into associations to better access market opportunities.

A special category of NGOs is those responding to emergency situations, such as the World Food Programme (WFP) which has already included biofortified crops in its procurement policy, as mentioned above. Linking producers of biofortified crops to the WFP and other organizations engaged in serving refugee and internally displaced populations serves the dual goal of reaching one of the most nutritionally vulnerable population groups while concurrently creating market opportunities (i.e., demand pull) nationally or regionally. Support is typically needed for smallholder farmers to be able to meet the quality criteria required for such markets.

Towards an improved food system

The global COVID-19 pandemic of 2020 has vividly exposed the weaknesses in food systems. With rising unemployment, and increasing cost of perishable foods (i.e. nutrient rich animal-sourced foods, fresh fruits and vegetables) as a result of disruptions to food supply chains, food and nutrition insecurity has increased in most countries. Even in developed countries, lower-income, vulnerable populations were especially affected and not surprisingly, under such conditions they heavily depend on cheaper and non-perishable staple foods for the vast majority of their energy needs. Clearly, biofortified staples have an increased importance in such settings and should be part of the longer-term strategy for building an improved food system, in which access to an affordable set of diverse foodstuffs for all is in ultimate goal (Heck et al. 2020).

Another crisis, namely climate change, is also having an increasingly growing negative impact on food and nutrition security. Not only is climate change creating greater fluctuations / uncertainties in productivity, often resulting in local or national food insecurity, but it is also affecting the nutrient content of commonly consumed staples with increasing GHG emissions decreasing the nutrient density of most plants (Smith et al. 2018). As a result, the gap between the demand for and supply of micronutrients is increasing (Nelson et al. 2018). Biofortified staples—majority of which are also bred to be climate smart (e.g., drought and flood resistant; heat tolerant, etc.)—with their higher micronutrient densities—which more than offset for this nutrient loss—could help in the efforts to improve food and nutrition security in the face of climate crises.

Chapter 6. The Potential of Transgenic Approaches in Biofortification

Genetic Engineering as a Tool to Create Biofortified Crops

Genetic engineering (GE), also known as genetic modification (GM), is a technology that allows introduction of adjustments as well as additions to the genetic code of an organism. Using this set of techniques, beneficial traits such as enhanced crop disease resistance or an enhanced micronutrient content can be implemented. GE can be performed from the smallest scale—modifying one letter of the genetic code—to a larger scale, where a whole set of genes is introduced. GE can thus be used to create biofortified crops with an enhanced content of minerals and vitamins. GE is complementary to traditional breeding since it can be used to introduce new traits into crops developed by breeding. As an example, the recently developed high iron, high zinc bean (Haas et al. 2016) could be further improved using GE to include traits such as disease resistance (Bonfim et al. 2007).

GE has several advantages over traditional breeding (listed in Table 8). First, GE allows the introduction of a trait that is absent in a tissue of interest (tissue specificity). When a new trait (e.g., enhanced provitamin A content) is desired, breeding requires the variation of this trait within the crop species. To introduce vitamin A into rice by breeding, a rice plant that already contains vitamin A in the kernel would be needed to backcross into a commercial variant. Since no such rice has yet been identified, conventional breeding cannot be used to develop vitamin A enriched rice, a trait which is relatively easy to achieve by GE (Ye et al. 2000).

Second, GE allows the introduction of a new *set* of genes—coming from other crops, a variant of the same crop, or even from outside the plant kingdom—into a single variety at once. In this way, new traits can be introduced in a crop more quickly (e.g., enhanced provitamin A, iron, and zinc content in rice (Singh et al. 2017a), requiring a much smaller number of crop generations than conventional breeding. GE thus brings new opportunities while saving a tremendous amount of time in product development. Moreover, GE does not hold the risk to include untargeted genes into the created product, as it involves introduction of a (set of) well-characterized gene(s).

However, GE requires sufficient fundamental knowledge of the plant's micronutrient metabolism before a solid GE strategy can be developed (Strobbe et al. 2018). This means that although many biochemical pathways have been unrav-

Table 8. Comparison between breeding and genetic engineering (GE) to be used for micronutrient biofortification of crops.

	Issue	GE	Breeding
Comparative Advantages of GE	Tissue-specificity	Ability to control tissue-specificity	No adequate control on tissue-specificity
	Source of genetic material	Introduction of genes across species barrier possible	Restricted to sexually compatible gene pool
	Time-consuming	Results obtained in limited number of generations	Requires many generations
	Transfer of untargeted genes	Transfer of well-defined genes	Potential transfer of multiple (untargeted) genes
Comparative Advantages of Breeding	Knowledge on metabolic pathways	Requires sufficient knowledge of metabolic pathways	Knowledge of metabolic pathways not required
	Enhanced knowledge on micronutrient metabolism	Limited potential to discover new genes involved	Ability to reveal new genes involved

Note: Aspects of both breeding and GE are listed. Note that the importance of these aspects can vary depending on the crop/micronutrient combinations. In some cases, a combined methodology, utilizing both breeding and GE, can be advised.

eled, there can be a higher entry cost compared to traditional breeding. The latter has the ability to reveal the existence of novel genes involved in micronutrient metabolism. Potential discovery of new genes is less likely in GE approaches. Nonetheless, GE brings many benefits and has supported several success stories already.

It stands to reason that enhanced nutrient densities in GM biofortified crops have an equally good nutritional value as conventionally bred crops. Taking provitamin A enriched rice as an example (see below for more information), efficacy to was shown in a clinical trial and several preliminary studies (De Moura et al. 2016; Tang et al. 2009). Another example is the case of folate enriched rice by GE, that was as successful in supplementing vitamin B9 as rice supplemented by folic acid in rats (Kiekens et al. 2015). For a more complete discussion we refer to (De Steur et al. 2017b). Despite the demonstrable benefits of GM biofortified crops their release to farmers has not been approved in many countries (De Steur et al. 2015). This is partly due to a very strict regulation of GM technology in many countries combined with anti-GM activism, but also partly explained by a lack of strategic design and follow-up for deployment of GM biofortified crops (Hefferon, 2015; Lucht, 2015; Napier et al. 2019). More recent biofortification efforts, as for instance iron-zinc enriched rice developed by researchers at the International Rice Research Institute (IRRI), seem to have learned from these pitfalls and developed the iron-zinc trait in a variety that is directly crossable with many commercial varieties (Trijatmiko et al. 2016).

Critics of transgenics often identify GE with industrialized, high-input farming approaches, unbeneficial for small farms and and harmful to the environment. However, plant breeding in general, and GE in particular, can in some cases significantly lower fertilizer and pesticide use, and improve water use efficiency. Higher yields allow for a reduction in area planted required to attain a given food output level, consequently limiting expansion of agricultural land use. GE is compatible with small-scale sustainable farming. If regulatory costs could be reduced, more public and private resources would be devoted to helping small-holder farmers.

Case Examples for GE in Biofortified Crops: Single Micronutrients and Multi-Biofortification

Crop biofortification using GE has been successfully achieved in a range of crops for many different nutrients. Three examples of micronutrient enhancement in main staple crops (wheat, rice, maize, cassava, potato) are highlighted below. A compilation of recent research on biofortification in different crops is presented in Garg and colleagues (2018).

Provitamin A

Golden rice (GR) is a famous example of provitamin A biofortification by GE. Two biosynthetic genes, PSY and CRTI, were introduced into rice to create Golden Rice. The PSY gene originates from maize while CRTI originates from bacteria.

The provitamin A content of rice was increased from zero to 3700 µg provitamin A per 100 gram dry rice (Paine et al. 2005; Ye et al. 2000). Introgression of the provitamin A trait into commercial Indica varieties resulted in rice which contains between 450 µg and 1100 µg provitamin A per 100 gram dry rice (Swamy et al. 2019). Considering potential loss due to cooking and a conservative conversion factor of the carotenoids to vitamin A, 300 g Golden Rice (±1,000 kcal) would provide around 33% of the vitamin A RDA for pregnant women (Swamy et al. 2019). Other crops in which provitamin A content was increased include bananas, cassava, maize, potatoes, and wheat. Since its development 20 years ago, the implementation of Golden Rice has been the subject of heavy debate. The recent approval by FDA and studies showing the potential health effects of Golden Rice (Owens 2018; Tang et al. 2009;), however, seem to have finally opened the door for the introduction of the biofortified rice in countries such as Bangladesh (Stokstad 2019), in which a large portion of the population is vitamin A deficient.

Iron (and zinc)

Cassava—also known as manioc or yuca—is a major staple in countries such as Nigeria, Mozambique, and Indonesia. Cassava contains up to 7 µg/g iron and 10 µg/g zinc per gram dry weight in the storage root. Researchers succeeded in developing cassava with an 18-fold increase in iron levels and a 10-fold increase in zinc levels through GE (Narayanan et al. 2019). Cassava was biofortified using two genes, one that enhances iron uptake and transport (IRT) and a second one that captures iron in the desired tissue (ferritin). Using a similar strategy, iron levels were also enhanced in rice (Trijatmiko et al. 2016) and wheat (Singh et al. 2017b).

In the example above and considering potential losses due to cooking and processing and bioavailability, 350 grams fresh weight of biofortified cassava (560 kcal) provides around 100% of the estimated average requirement for iron for nonpregnant, nonlactating women while consumption of 110 grams (176 kcal) provides around 100% of the EAR for children between 4 and 6 years old (Narayanan et al. 2019).

Folates (vitamin B9)

Vitamin B9 is a water soluble vitamin, consisting of different folate forms. Folate deficiency has been causally linked with severe birth defects and different forms of anemia (McLean et al. 2008). Primarily children and pregnant women are susceptible to folate deficiency. A minimum estimate of incidence is around 300,000 births annually affected by neural tube defects, the majority of which is due to inadequate maternal folate intake (Zaganjor et al. 2016). This is probably a severe underestimation, given lack of surveillance systems in low and middle income countries.

Folate biofortification has been successfully achieved in several staple crops. Prime examples are rice and potato. In both cases, the endogenous folate biosynthesis was boosted by expression of several *Arabidopsis* (thale cress) plant folate genes (*GTPCH* and *ADCS* for rice; *GTPCH, ADCS* and *HPPK/DHPS* for potato) combined with the stabilization of folates by the expression yet another *Arabidopsis* gene (*FPGS*). Note that these strategies only make use of the genetic variety of the plant kingdom (Blancquaert et al. 2015; De Lepeleire et al. 2018).

Folate levels were enhanced from 18 µg to 1700 µg per 100g of dry milled rice and from 33 µg to 385 µg per 100 g of potato (Blancquaert et al. 2015; De Lepeleire et al. 2018). This means that in order to obtain the recommended daily allowance (RDA) for folates solely from rice consumption, considering potential losses due to cooking and processing, consumption of around 150 g of dry milled biofortified rice (±500 kcal) would be sufficient.

Simultaneous Increase of Multiple Micronutrients

An obvious limitation of using conventional breeding for biofortification is the slow process of breeding in a single mineral or vitamin at a time sequentially, a process which is also constrained by using variation in available germplasm. Several minerals and vitamins are lacking in diets in LMICs in any given country. GE allows trait stacking into a single genomic insert, harboring combined genetic elements, which can greatly speed up the process of putting multiple micronutrients in the same variety of a staple food crop, referred to as multi-biofortification. Implementation of multi-biofortification further improves the cost-effectiveness of the nutritional intervention, as several nutrient deficiencies can be addressed simultaneously (De Steur et al. 2017a).

One of the first successful examples involves multi-vitamin corn, in which higher levels of provitamin A (59 µg/g dry weight β-carotene, 169-fold increase), vitamin C (107 µg/g dry weight, 6-fold increase) and folate (2 µg/g dry weight, 2-fold increase) were reached making use of genetic engineering technology (Naqvi et al. 2009). More recently, transgenic multi-nutrient biofortified rice was obtained, which exhibited significantly greater levels of iron, zinc as well as provitamin A (Singh et al. 2017a). This is a nice example of a combination of previous knowledge on rice biofortification of provitamin A (Paine et al. 2005) as well as of iron/zinc (Wirth et al. 2009).

Similarly, transgenic multi-biofortification of sorghum resulted in highly elevated levels of the fat-soluble vitamins A and E (Che et al. 2016), exhibiting an 18-fold (9.3 µg/g dry weight β-carotene) increase in provitamin A as well as a 1.8-fold (3 µg/g dry weight) increase in α-tocopherol when compared to control plants. Interestingly, the higher levels of the antioxidant (vitamin E) provided protection against oxidative breakdown of provitamin A compounds, resulting in higher provitamin A stability, greatly extending its shelf-life (Che et al. 2016). This demonstrates how increasing vitamin levels holds the potential to have beneficial effects on food quality beyond the obvious nutritional benefit of the increased micronutrient level.

On top of these positive effects for human nutrition, increasing antioxidant levels in crops could increase their resilience to environmental stresses. Indeed, enhanced antioxidant levels in the form of vitamins B6 and C, have shown to positively impact general growth and stress tolerance in the model plant Arabidopsis, respectively (Raschke et al. 2011; Zhang et al. 2012). These insights have enabled implementation of these endeavors in food crops, giving rise to high ascorbate tomatoes and B6 accumulating potatoes, both exhibiting higher tolerance to salt stress (Bagri et al. 2018; Zhang et al. 2011).

Combining Agronomic Traits With Nutrition Traits and Use of More Recent Gene Editing Tools

Current and future research aims to harness the full potential of transgenic multi-nutrient biofortification strategies by incorporation of agronomic traits, increasing the crop's potential from an economic point of view, while also utilizing the possibilities provided by novel genome editing tools where applicable (Figure 4). Desired traits such as pest resistance (Tabashnik and Carriere 2017) and drought tolerance (Castiglioni et al. 2008) would aid in increasing crop yield as well as resilience to abiotic and biotic stresses, which is required to cope with population increase and climate change (Bailey-Serres et al. 2019; Beacham et al. 2018).

Genome editing tools (e.g., CRISPR/Cas (Shan et al. 2013)) allow targeted insertion of the genetic material (e.g. DNA sequence containing genetic information to allow vitamin and mineral enhancement as well as agronomic crop improvement) at a predetermined location on the genome of the crop of interest. In a recent

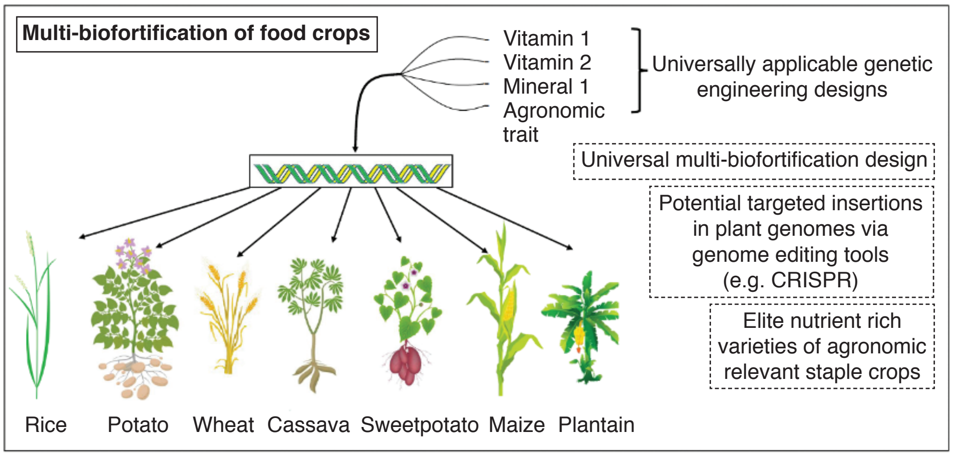


Figure 4. Multi-biofortification of food crops. Genetic engineering strategies are perfected to deliver multiple vitamins as well as minerals. Interesting agronomic traits (e.g. higher yield) can be included in micronutrient biofortification designs. In ideal cases, these designs can be universally applicable, with relatively minor modifications, in an array of important food crops. Genome editing tools can be used to guide the genomic insertion to a well-characterized safe-harbor region in the crop genome. In doing so, resilient nutritious crops of high agronomic value can be obtained.

proof-of-concept study in rice, the genetic information required for provitamin A enhancement in seeds was delivered in this way, reaching carotenoid levels in seeds similar to previous transgenic methods (β-carotene content up to 7.9 µg/g dry weight)(Dong et al. 2020). Moreover, this also eliminated the need for plant selectable markers, which was previously required in the creation of transgenic crops. The latter has been considered a downside of GE as this often involved additional incorporation of herbicide tolerance genes into the genomic DNA insert (Zhang et al. 2016).

Furthermore, genome editing tools can be utilized to make minute genomic alterations (e.g. changes one DNA nucleotide), though they cannot be considered the panacea to solve all problems. The methodology was shown to improve provitamin A content in rice callus (undifferentiated tissue), without transgene insertion (Endo et al. 2019). In this way, genome editing technology can, in some specific cases, eliminate the need to introduce foreign genetic elements to achieve a satisfactory micronutrient level. However, it remains to be demonstrated that the latter approach is effective to stimulate the carotenoid metabolism in rice seeds. In depth research will be necessary to identify the genetic elements that can serve to boost gene transcription to levels unprecedented under normal conditions.

Transferring successes from one crop to another

A specific transgenic (multi)biofortification strategy can be readily transferred from one crop species to an array of crops (Figure 4). This is illustrated by the case of folate biofortification, in which the same two-gene strategy was used, after minimal modifications, to increase folate content in rice (Storozhenko et al. 2007), tomato (de La Garza et al. 2007), wheat, and corn (Liang et al. 2019). However, extrapolation to a different crop could prove to be difficult, as the aforementioned folate biofortification strategy appeared less effective in potato tubers (Blancquaert et al. 2013), which was ameliorated by addition of an auxiliary gene (De Lepeleire et al. 2018). Therefore, implementation of a (multi) biofortification design should be validated and fine-tuned on a crop-specific basis, whenever needed.

Conclusion

As for conventionally-bred biofortified crops, retention, bioavailability, and efficacy of added minerals and vitamins must be established in GE-developed varieties, as well as undertaking multilocation field trials that prove the potential for high agronomic performance to make adoption attractive to farmers. More rapid deployment of GE-developed crops continues to be hampered severely by inefficient and in some cases unnecessary regulatory barriers (Van Der Straeten et al. 2020). Fostering positive public perception through open and unbiased communication and maximizing public funding of biofortified staples, as well as offering added value for farmers, could facilitate deployment of GE-crops (Napier et al. 2019).

Future multi-biofortification endeavors should rationally combine the potential and advantages of GE with genome editing tools and conventional breeding to maximize impact. Hence, plant breeding projects can further provide agronomically important plant lines as well as fundamental knowledge, if combined with transgenic multi-biofortification to create desired nutritionally complete crops. These high micronutrient crops will be a valuable tool to be used, in concert with dietary education and supplementation, to eradicate micronutrient malnutrition as part of the United Nations' sustainable development goal 2 (UN-SDG2-zero hunger).

Chapter 7.
Summary and the Way Forward

Summary

When the concept of biofortification was first broached in the 1990s there was much skepticism that implementation would be feasible and that biofortification could make a significant public health impact. In view of the published experimental evidence, the experience in practical imple-mentation gained over the past decade, and advocacy and communications efforts, biofortification has now been accepted as a recognized and important intervention in the fight against mineral and vitamin deficiencies (HarvestPlus 2020d).

The proof of concept of biofortification, as laid out in the three primary questions posed Chapter 1, has been demonstrated. The levels of iron, zinc, and provitamin A in released biofortified crops, tested under controlled conditions (efficacy trials), are high enough and sufficiently bioavailable to show improvements in mineral and vitamin status, and better functional/health outcomes. For pro-vitamin A sweetpotato, an effectiveness study covering farm populations both in Mozambique and Uganda demonstrated impact at scale, when combined with a nutrition education component (Chapter 2).

These levels of nutrients have been bred into high-yielding, climate-smart and profitable varieties that are attractive to farmers. Breeding pipelines have been established with varieties with higher mineral and vitamin densities still to be released. Biofortified varieties have been released in 40 countries, and are now adopted by 15 million farm households (Chapter 3).

Delivery strategies have been developed and tested for all kinds of biofortified crops including hybrid, self-pollinated, and vegetatively-propagated to cost-effectively integrate biofortified seeds and foods into seed and food systems in specific countries. For vitamin A crops, the change in color from white to yellow/orange due to provitamin A was proven not to be a significant barrier to con-sumer demand (Chapter 4), in fact the color could be used for promotion strategies; while for mineral crops it was highlighted that branding and certification mechanisms would be needed. Several types of institutions, operating all along the value chain, have begun to promote and undertake biofortification as part of their core activities (Chapter 5).

This evidence supports the ex ante assumptions made in benefit-cost studies reviewed in Chapter 1, supporting a conclusion that biofortification is one of most cost-effective interventions available in the campaign to reduce mineral and vitamin deficiencies. Chapter 6 discusses the potential for genetic engineering to significantly multiply the impacts possible using conventional breeding.

Reaching SDG #2 Goals and Dietary Quality Under the COVID-19 Pandemic

Attaining the SDG #2 goal of ending hunger will require a significant improvement in the diets of the poor. A fundamental component of future food systems are food staples which provide a base of significant levels of several essential minerals, vitamin, and other nutrients as demonstrated by and discussed for the Philippines example in Chapter 1. *Substituting* biofortified foods for non-biofortified crops, one-for-one, involves no requirement for increased food expenditures. Because they are high-yielding, biofortified foods will sell for the same price as non-biofortified foods—and of course at the same time provide additional nutrients.[8]

Those undertaking the important work of improving food systems to delivery healthier diets will focus on increasing the *quantities* consumed of key nutrient-rich foods, such as fresh fruits and vegetables and animal sourced-foods. Those *purchasing* higher quantities of these nutrient-rich foods will be required perforce to increase their food expenditures, requiring increases in income. Both strategies will contribute significantly to meeting the goal of improving diets to end malnutri-tion in all its forms, including the hidden hunger.

The evidence shows that biofortification is efficient, cost-effective, and deliverable for improving human nutrition *in normal times*. The threats from global climate change, soil loss, and water de-pletion, raise another question—in improving health and nutrition, how *resilient* is biofortification, compared to alternatives? An ongoing test is provided by the COVID-19 pandemic.

Under the current pandemic, it is well-documented that incomes have fallen, along with the supply chain disruptions and hence availability of nutrient-rich foods, making them unaffordable to the poor whose dietary quality has therefore significantly worsened (FAO 2020). Very likely intakes of animal-sourced foods, as well as pulses, fruits, and vegetables, are declining, as it was seen during the food price crises of 2008, which then lead to significant increases in micronutrient deficiencies (Christian 2010). In contrast, consumption of food staples does not fall significantly in bad times (except under severe famine conditions), and does not rise significantly in good times. Government policies give high priority to ensuring that adequate supplies of food staples are available to keep food staple prices from rising and potentially volatile urban populations free from explicit hunger.

The COVID-19 pandemic coupled with the ongoing climate crises has served to focus attention on the need for resilient, sustainable food systems that provide nutritious diets at affordable prices. Given the affordability and accessibility of staples, their biofortification can contribute substantially to micronutrient resiliency, locally and cost-effectively. This paper has summarized the evidence and knowledge to date on the feasibility, efficacy and scalability of staple food biofortification. Based on this evidence and knowledge base, we call for continued investment in biofortification science and for multisectoral action to mainstream biofortification in food systems to help achieve nutritious, affordable and sustainable diets for all.

The Way Forward

Finally, we identify scientific and policy issues that will determine the extent to which biofortification will realize its full potential for contributing to the reduction in malnutrition in LMICs. We aim to contribute to framing future discussions, not to provide in-depth analysis and answers. First, we discuss issues internal to the implementation of biofortification itself, and then we turn to the context of biofortification as one intervention in the larger effort to coordinate nutrition-direct and nutrition-smart food systems interventions to reach SDG #2 goals.

Issues Internal to the Implementation of Biofortification

What additional nutrition qualities/attributes can/should be packed into the edible por-tions of staple foods without sacrificing yield?

[8] In the short-run, if demand for a biofortified food is high and supplies are limited, the price of a biofortified food may be relatively high. However, the higher price will elicit a supply response which will bring the price down in equilibrium. For example, basmati rice is relatively expensive due to its lower yield.

What should be our food staple nutrition breeding objectives?

For decades, food staples will remain an essential source of a range of nutrients for the poor (Chapter 1). Initial efforts at biofortification have focussed on increasing the density of iron, zinc, and provitamin A in food staples. However, Chapter 2 raised the issue briefly of breeding for compounds in food staples that improve gut health and increase the bioavailability of a range of trace minerals. There is already substantial work on folates (Chapter 6). HarvestPlus is evaluating the feasibility of breeding for calcium. IRRI is developing rices with a low glycemic indices which could lower the probability of developing diabetes. Breeding strategies for reducing aflatoxins in maize have been initiated. Optimizing the nutritional and health impacts of food staples holds a myriad of opportunities.

Work to improve nutrition through food staples must focus on bioabsorbable nutrient density, not quantity consumed. However, two forces currently work in the opposite direction—to *lower the nutrient densities in staple foods*. For decades plant breeding for food staples, such as wheat, has sought to increase yield and starch content without regard to nutrient content. As a result, wheat has lost approximately 0.7% of total protein and approximately 10 ppm iron and zinc dry matter. (Shewry, Pellny, and Lovegrove 2016). Historical trends have not been carefully studied for a range of nutrients and crops.

Second, experiments have shown that rising levels of CO_2 in the atmosphere, due to climate change, increase the growth of cereal crops, but lower mineral and vitamin densities in the seeds of food staples (Ebi and Ziska 2018; Myers et al. 2014)

Current efforts to breed just for higher densities of iron, zinc, and provitamin A must be successful, net of these two trends. It is fortunate that genetic engineering methods are potentially available to deal more efficiently with maintaining and increasing a range of nutrients and compounds in single varieties of staple food crops (Chapter 6).

Will "mainstreaming" of biofortification at agriculture research centers be successfully integrated into core activities over the long-term?

In 2003, agricultural donors and CGIAR Center managers took a conservative approach to biofortification. At each Center, separate breeding pipelines were set up for biofortified crops, which were not initially included in the main Center breeding programs, an approach referred to as targeted breeding. The view was once it is proven that biofortification can have significant impacts, min-eral and vitamin traits will be mainstreamed into Center breeding programs.

Mainstreaming has now begun for zinc for rice and wheat, but may take a decade and will require investment, perseverance, and priority. In retrospect, integrating biofortification into core breeding programs has taken too long. Moreover, targeted breeding needs to continue as a bridge until mainstreaming is fully implemented. A promising development is that sweetpotato breeders from 14 national programs in sub-Saharan Africa signed a statement committing to at least 50% of the cultivars being presented to release committees would be orange-fleshed.

Progress in breeding is a stepwise process. Under mainstreaming, which nutrition-enhancing and yield-enhancing traits will be prioritized? It is critical that agricultural scientists continue to work with nutritionists and be informed about the benefits of biofortification.

What will be the future role of genetic engineering methods?

Genetic engineering methods can significantly reduce the gestation times for agriculture research. Individual traits can be added more quickly, and multiple traits can be stacked simultaneously. In addition, certain traits that cannot be obtained by breeding, can be introduced by genetic engineering, such as provitamin A in rice seeds.

However, fears of genetically-engineered foods and inefficient and sometimes unnecessary regulatory restrictions continue to constrain the use of this powerful and potentially beneficial technology. Research should continue, while a more intensified effort is needed to address consumer and policy maker concerns about genetically engineered products.

What will be the role of consumer demand?

White is the current norm for the color of food staples in LMICs, such as for cassava, maize, and rice. Can orange/yellow become the new norm? As long as there is price equality between white and orange/yellow, the value proposition to families is very strong. However, governments and other institutions will have to invest and strongly back the message that yellow/orange is healthier.[9] If orange/yellow becomes the new norm, consumer demand would ensure that the food systems supply high provitamin A food staples.

This may also create awareness for high (but invisible) mineral content. However, rather than establishing separate supply chains and labelling biofortified and non-biofortified foods—and instituting and enforcing regulations for biofortified foods—the most efficient path would be to seek that all staple foods are biofortified with iron and zinc, just as universal fortification of a commercial food is the least expensive strategy.

But we also need to actively promote and market biofortified crops (and other healthy foods) with non-visible traits. Informed leaders, practitioners, and ultimately consumers are requisite for driving food system change. There is also a challenge in improved engagement not just with consumers, but traders and processors, so more attention is paid to understanding the nutrition needs and desires of different segments of the population. Any biofortified crop must be competitive yield- and taste-wise to compete with existing varieties.

How will funding for biofortification be coordinated efficiently?

In Chapter 1, we outlined how initial funding for biofortification was centralized in HarvestPlus, so that cross-disciplinary and institutional investments could be coordinated according to a central strategy within particular crops. Also with centralized funding, lessons most easily and efficiently could be learned and applied across crops within particular disciplines. Institutions prefer, however, not to be coordinated, but to receive funding directly from donors.

Now that biofortification is a "proven" strategy, individual institutions are applying for and receiving biofortification funding for individual component activities for specific crops, making coordination and sustained long-term funding much more difficult. However, a technically solid program or institution to continue resource mobilization, advocacy and policy input, and developing public-private sector partnerships particularly for seed, is warranted to ensure continued progress.

Issues of Coordination of Nutrition-Smart Agricultural Interventions with Complementary Nutrition Direct Interventions

Biofortification is but one nutrition-smart agricultural intervention.

To what extent can agreement and coordination be achieved on providing the most cost-effective mix of interventions in any given country?

It is critical that we move away from the either-or investment decisions advocated due to limited resource envelopes to address the complex challenge of providing affordable quality diets to all. Different interventions operate in different time frames, with agricultural interventions providing more sustainable and cost-effective solutions in the long run, impacting especially rural populations.

Capsule supplementation with key micronutrients will continue to be essential to address severe deficiencies in the short run. Industrial fortification can be cost-effective in providing multiple micronutrients to urban consumers in particular. Both these approaches incur the same recurrent annual costs.

Use of certain fertilizers (which may be implemented relatively rapidly) and improvement of soil health can improve the protein, iron, and zinc content of grains, and the beta-carotene content of sweetpotato, and these effects may be enhanced by fortifying fertilizers and foliar sprays with zinc, selenium, and iodine, an intervention known as agronomic biofortification. These effects of fertilizers and sprays may be synergistic with the use of biofortified crops (Zou et al. 2019).

To what extent are the long gestation periods of agriculture interventions to realize impacts and complexity of food systems barriers to sufficient investment?

There are many great ideas for agriculture to help fill the dietary quality gap. A long-term process has been described in previous chapters for but one successful example, biofortification. One important lesson that biofortification teaches is that policymakers and donors need to acknowledge at the outset that any specific agricultural strategy *will take decades to implement cost-effectively at scale across a number of countries*—a process that involves raising the funding, doing the research, building consensus, changing policies, securing uptake by farmers and acceptance by consumers, and so forth.

Food systems are complex. How does one choose where and how to intervene, especially when resources are constrained? "Improve Food Systems" is too broad and general a message. The focus should not be on particular bottlenecks across all foods, but on individual key foods themselves (which will vary by country), and then which bottlenecks need to be overcome across the value chain for that food to be scaled up or mainstreamed into the food system.

For example, dietary quality may be improved by increasing the national productivity of egg and milk production, and of particular vegetables and pulses in specific countries, thereby lowering prices, and increasing consumption of these foods. Small fish eaten whole (including bones, eyes, etc.) gram-for-gram are more nutritionally dense and less costly, than the fillets of larger fish.

New tools have been developed to monitor the cost of nutritious diets, and whether they are in reach of the poor (Herforth et al. 2020; Masters et al. 2018) and assuring that biofortified crops are included in such tools will enhance capturing progress and identifying bottlenecks.

To what extent will agricultural policymakers and agricultural donors give priority to nutrition-smart food systems?

Success in working productively with the nutrition community to improve dietary quality and to reduce malnutrition is premised on a high level of cooperation of agricultural policymakers and donors. However, the importance of a quality diet and the negative long-term impacts of poor diets is often unknown, or neglected by agricultural policymakers.

Often only implicitly, the primary reason that we produce food is, through its consumption, to be *healthy* and so have the ability to lead happy and productive lives. Explicitly, more often the motivations are to consume food to have energy (avoid hunger) and for pleasure. For most farmers, generating sufficient income from their production is an essential goal.

In LMICs, there is a policy focus on the price of food staples. Rising staple food prices can cause political instability. Keeping food staple prices low averts hunger, but reduces rural incomes and, therefore their nutritional status. Separate entities (typically agriculture and health) tackle production and consumption efforts separately. In a few countries, this has been addressed by multi-sector nutrition units bringing the different sectors together to coordinate planning and actions. However, the need to work across sectors to prioritize activities through a nutrition lens is given low priority in agricultural policymaking and investments.

A recent global study estimated that 3 billion people, concentrated in South Asia and sub-Saharan Africa, lack sufficient income to purchase the cheapest possible healthy diet recommended by national governments (Herforth et al. 2020). Agricultural production and food consumption–and so health, even cognitive ability–should not be addressed separately at the national level in LMICs.

Conclusion

Although substantial progress has been made, biofortification is not yet tightly woven into the fabric of present-day food

[9] For example, carrots used to be white and purple, but this is now forgotten. Consumer preferences can change dramatically over time.

systems as part of the core activities of a number of institutions (Chapter 5). Will the full potential of biofortification be realized? This depends on perseverance, investment, and leadership.

Awareness and efforts to link agriculture and food systems to human nutrition are more in evidence now than twenty, even ten years ago, but it is uncertain how this will play out and be sustained. It is important to show successes, but agricultural impacts develop slowly. Biofortification is in the forefront of demonstrating just how resilient, sustainable, and cost-effective agricultural interventions can be for improving nutrition and health.

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