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Biofortification: Progress Toward a More Nourishing Future[1]

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Abstract

Biofortification, the process of breeding nutrients into food crops, provides a sustainable, long-term strategy for delivering micronutrients to rural populations in developing countries. Crops are being bred for higher levels of micronutrients using both conventional and transgenic breeding methods; several conventional varieties have been released, while additional conventional and transgenic varieties are in the breeding pipeline. The results of efficacy and effectiveness studies, as well as recent successes in delivery, provide evidence that biofortification is a promising strategy for combatting hidden hunger. This review highlights progress to date and identifies challenges faced in delivering biofortified crops.

1. Justification

In the past 40 years, agricultural research for developing countries has focused on increased cereal production. Recently, there has been a shift: agriculture must now not only produce more calories to reduce hunger, but also more nutrient-rich food to reduce hidden hunger.[3] One in three people in the world suffer from hidden hunger, caused by a lack of minerals and vitamins in their diets, which leads to negative health consequences (Kennedy *et al.* 2003).

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[3] An important part of the overall solution is to improve the productivity of a long list of non-staple food crops. Because of the large number of foods involved, achieving this goal requires a very large investment, the dimensions of which are not addressed here.

Biofortification, the process of breeding nutrients into food crops, provides a comparatively cost-effective, sustainable, and long-term means of delivering more micronutrients. Biofortified staple foods cannot deliver as high a level of minerals and vitamins per day as supplements or industrially fortified foods, but they can help by increasing the daily adequacy of micronutrient intakes among individuals throughout the lifecycle (Bouis *et al.* 2011). Note that biofortification is not expected to treat micronutrient deficiencies or eliminate them in all population groups. No single intervention will solve the problem of micronutrient malnutrition, but biofortification complements existing interventions to sustainably provide micronutrients to the most vulnerable people in a comparatively inexpensive and cost-effective way (Bouis 1999; Nestel *et al.* 2006; Pfeiffer and McClafferty 2007; Qaim *et al.* 2007; Meenakshi *et al.* 2010).

Biofortification provides a feasible means of reaching malnourished rural populations who may have limited access to diverse diets, supplements, and commercially fortified foods. The biofortification strategy seeks to put the micronutrient-dense trait in those varieties that already have preferred agronomic and consumption traits, such as high yield. Marketed surpluses of these crops may make their way into retail outlets, reaching consumers in first rural and then urban areas, in contrast to complementary interventions, such as fortification and supplementation, that begin in urban centers.

Unlike the continual financial outlays required for supplementation and commercial fortification programs, a one-time investment in plant breeding can yield micronutrient-rich planting materials for farmers to grow for years to come. Varieties bred for one country can be evaluated for performance in, and adapted to, other geographies, multiplying the benefits of the initial investment. While recurrent expenditures are required for monitoring and maintaining these traits in crops, these are low compared to the cost of the initial development of the nutritionally improved crops and the establishment, institutionally speaking, of nutrient content as a legitimate breeding objective for the crop development pipelines of national and international research centers.

Currently, agronomic, conventional, and transgenic biofortification are three common approaches. Agronomic biofortification can provide temporary micronutrient increases through fertilizers. Foliar application of zinc fertilizer, for example, can increase grain zinc concentration by up to 20 parts per million (ppm) in wheat grain in India and Pakistan, but only in the season it is applied (Zou *et al.* 2012). This is nearly the full target increment set by nutritionists and sought in plant breeding (further described below). About half the target increment can be realized in rice; in maize, foliar application resulted in only a small effect (Phattarakul *et al*. 2012; Kalayci *et al*. 2011). This approach could complement plant breeding efforts but further research is needed.

Biofortification can be achieved through conventional plant breeding, where parent lines with high vitamin or mineral levels are crossed over several generations to produce plants that have the desired nutrient and agronomic traits. Transgenic approaches are advantageous when the nutrient does not naturally exist in a crop (for example, provitamin A in rice), or when sufficient amounts of bioavailable micronutrients cannot be effectively bred into the crop. However, once a transgenic line is obtained, several years of conventional breeding are needed to assure that the transgenes are stably inherited and to incorporate the transgenic line into varieties that farmers prefer. While transgenic breeding can sometimes offer micronutrient gains beyond those available to conventional breeders, many countries lack legal frameworks to allow release and commercialization of these varieties.

Implementing Biofortification

For biofortification to be successful, three broad questions must be addressed:

– Can breeding increase the micronutrient density in food staples to target levels that will make a measurable and significant impact on nutritional status?

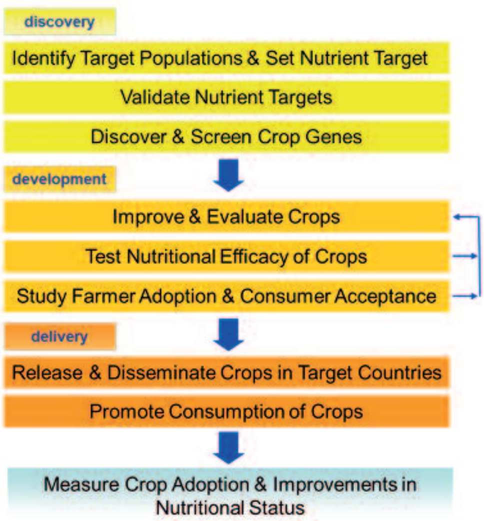

Figure 1. HarvestPlus Impact Pathway.

– When consumed under controlled conditions, will the extra nutrients bred into the food staples be absorbed and utilized at sufficient levels to improve micronutrient status?
– Will farmers grow the biofortified varieties and will consumers buy/eat them in sufficient quantities?

To answer these questions, researchers must carry out a series of activities classified in three phases of discovery, development, and dissemination. This impact pathway is illustrated in Figure 1 and discussed in greater detail in Bouis *et al.* (2011).

Discovery

The overlap of cropping patterns, consumption trends, and prevalence of micronutrient malnutrition, as well as *ex ante* cost-benefit analyses, determine target populations and focus crops. Nutritionists then work with breeders to establish nutritional breeding targets. These target levels take into account the average food intake and habitual food consumption patterns of target population groups, nutrient losses during storage and processing, and nutrient bioavailability (Hotz and McClafferty 2007).

Under HarvestPlus, breeding targets are set such that, for preschool children 4-6 years old and for non-pregnant, non-lactating women of reproductive age, the incremental amount of iron will provide approximately 30 percent of the Estimated Average Requirement (EAR), that incremental zinc will provide 40 percent of the EAR, and that incremental provitamin A will provide 50 percent of the EAR. Bioavailability of iron was originally assumed to be 5 percent for wheat, pearl millet, beans, and maize (10 percent for rice, cassava, and sweet potato), that of zinc 25 percent for all staple crops, and for provitamin A 8.5 percent for all staple crops (12 molecules of beta-carotene produce 1 molecule of retinol, the form of vitamin A used by the body).

Plant breeders screen existing crop varieties and accessions in global germplasm banks to determine whether sufficient genetic variation exists to breed for a particular trait. Initial research indicated that selection of lines with diverse vitamin and mineral profiles could be exploited for genetic improvement (Dwivedi *et al.* 2012; Gregorio 2002; Ashok Kumar *et al.* 2012; Velu *et al* 2012; Gomez-Becerra *et al.* 2010; Maziya-Dixon *et al.* 2000; Talukder *et al.* 2010; Jiang *et al.* 2008; Fageria *et al.* 2012; Menkir 2008; Beebe *et al.* 2000; Monasterio and Graham 2000; Menkir *et al.* 2008). Genetic transformation is an alternative method to incorporate specific genes that express nutritional density.

Development

Crop improvement includes all breeding activities. Initial product development is undertaken at international research institutes to develop varieties with improved nutrient content and high agronomic performance, as well as preferred consumer qualities. When promising high-yielding, high-nutrient lines emerge, they are tested by national research partners and the best-performing lines then selected to submit to national governments for release. The formal release process varies by country, but in general requires that a variety be grown and evaluated in several different locations (called multilocational trials) for at least two seasons, and its performance compared to other candidate and widely released varieties, before the national government approves the variety for dissemination. The breeding, testing, and release process can take 6 to 10 years to complete.

Parallel to crop improvement, nutrition research measures retention and bioavailability of micronutrients in the target crop under typical processing, storage, and cooking practices. Initially, relative absorption is determined using in vitro and animal models and, with the most promising varieties, by direct study in humans in controlled experiments. Randomized, controlled efficacy trials demonstrating the impact of biofortified crops on micronutrient status and functional indicators of micronutrient status (i.e. visual adaptation to darkness for provitamin A crops, physical activity for iron crops, etc.) provides evidence to support biofortified crops as alternative public health nutrition interventions.

Economics research on consumer and farmer evaluation of biofortified varieties, as well as varietal adoption studies, further informs crop improvement research during the development phase.

Dissemination

Biofortified crops must be formally released in the target countries prior to their delivery to the target populations. Economists lead consumer acceptance, varietal adoption, and seed and grain value chain studies to inform effective, efficient, and targeted delivery and marketing strategies to maximize adoption and consumption of these crops.

2. Current Status of Biofortified Crops

HarvestPlus leads a global interdisciplinary alliance of research institutions and implementing agencies in the biofortification effort. The Bill and Melinda Gates Foundation-funded Grand Challenges 9 is developing several transgenic crops. Progress made with key crops is reviewed, summarized in Figure 2.

CROP	NUTRIENT	TARGET COUNTRY	LEAD INSTITUTIONS	FIRST RELEASE YEAR
Banana/Plantain	Provitamin A Carotenoids	Nigeria, Ivory Coast, Cameroon, Burundi, DR Congo	IITA, Bioversity	Unknown
	Provitamin A Carotenoids, Iron*	Uganda	Queensland University of Technology, NARO	2019
Bean	Iron (Zinc)	Rwanda, DR Congo	CIAT, RAB, INERA	2012
		Brazil	Embrapa	2008
Cassava	Provitamin A Carotenoids	DR Congo	IITA, CIAT, INERA	2008
		Nigeria	IITA, CIAT, NRCRI	2011
		Brazil	Embrapa	2009
	Provitamin A Carotenoids, Iron*	Nigeria, Kenya	Donald Danforth Plant Science Center	2017
Cowpea	Iron, Zinc	India	G.B. Pant University	2008
		Brazil	Embrapa	2013
		China	Institute of Crop Science, YAAS	2015
		India	DBT	Unknown
Pearl millet	Iron (Zinc)	India	ICRISAT	2012**
Pumpkin	Provitamin A Carotenoids	Brazil	Embrapa	2015
Rice	Zinc (Iron)	Bangladesh, India	IRRI, BRRI	2013
		Brazil	Embrapa	2014
	Provitamin A Carotenoids*	Philippines, Bangladesh, Indonesia, India	Golden Rice Network, IRRI	2014
	Iron*	Bangladesh, India	University of Melbourne, IRRI	2022
	Iron	China	Institute of Crop Science, CAAS	2010
Sorghum	Zinc, Iron	India	ICRISAT	2015
	Provitamin A Carotenoids*	Kenya, Burkina Faso, Nigeria	Africa Harvest, Pioneer Hi-Bred	2018
Sweet potato	Provitamin A Carotenoids	Uganda	CIP, NaCCRI	2007
		Mozambique	CIP	2002
		Brazil	Embrapa	2009
		China	Institute of Sweet Potato, CAAS	2010
Wheat	Zinc (Iron)	India, Pakistan	CIMMYT	2013**
	Zinc (Iron)	China	Institute of Crop Science, CAAS	2011
	Zinc (Iron)	Brazil	Embrapa	2016

For projected releases, "first release year" refers to the first country listed.

* Denotes transgenic variety

** Denotes commercialization year; official release in subsequent year

( ) Denotes secondary nutrient

BRRI: Bangladesh Rice Research Institute; CAAS: Chinese Academy of Agricultural Sciences; CIAT: International Center for Tropical Agriculture; CIMMYT: International Maize and Wheat Improvement Center; CIP: International Potato Center; DBT: Department of Biotechnology; IAR&T: Institute of Agricultural Research and Training; ICARDA: International Center for Agricultural Research in the Dry Areas; ICRISAT: International Crops Research Institute for the Semi-Arid Tropics; IITA: International Institute of Tropical Agriculture; INERA: Institut National pour l'Etude et la Recherche Agronomiques; IRRI: International Rice Research Institute; NaCRRI: National Agricultural Crops Resources Research Institute; NARO: National Agricultural Research Organisation; NRCRI: National Root Crops Research Institute; RAB: Rwanda Agriculture Board; YAAS: Yunnan Academy of Agricultural Sciences; ZARI: Zambia Agriculture Research Institute.

Figure 2. Biofortified Target Crops and Countries-Release Schedule.

2.1 Conventional Breeding

Orange Sweet Potato (OSP)

After screening identified varieties that twice exceeded the target level of 30 ppm of provitamin A, varieties were improved by the International Potato Center (CIP) and National Agriculture Research and Extension System (NARES) scientists to suit local tastes and agronomic conditions.

Nutrition research indicated that provitamin A retention was greater than 80 percent after boiling or steaming and at least 75 percent after solar or sun drying, typical types of preparation (van Jaarsveld *et al.* 2006; Hagenimana *et al.* 1999; Wu *et al.* 2008; Bechoff *et al.* 2010; Bengtsson *et al.* 2008; Kimura *et al.* 2006). Provitamin A from OSP is highly bioavailable and its consumption can result in a significant increase in vitamin A body stores across age groups (Haskell et. al 2004; Jalal *et al.* 1998; Low *et al.* 2008; van Jaarsveld *et al.* 2005).

Mozambique and Uganda released high-provitamin A varieties in 2002 and 2007, respectively. Biofortified varieties are now being introduced in many parts of Africa and South America, as well as China. In 2009, CIP launched its Sweetpotato for Profit and Health Initiative (SPHI), which seeks to deliver OSP in Africa to reach 10 million households by 2020. The project focuses on empowering women farmers, expanding market opportunities, diversifying the use of sweet potato, and enhancing the breeding pipeline for OSP varieties. Helen Keller International (HKI) has integrated biofortification into its programs to combat vitamin A deficiency, promoting OSP through nutrition education coupled with homestead food production. HKI works with CIP on the Reaching Agents of Change project, which seeks to leverage increased investment in OSP across Africa.

Maize

Provitamin A maize breeding is led by International Maize and Wheat Improvement Center (CIMMYT) and International Institute of Tropical Agriculture (IITA) in conjunction with NARES in southern Africa. Germplasm screening discovered genetic variation for the target level (15 ppm) of provitamin A carotenoids in temperate maize, which was then bred into tropical varieties. Recent developments in marker-assisted selection technology have increased the speed and accuracy of identifying genes controlling the traits of interest in maize. Varieties that can provide 25 percent of the EAR for adult women and preschool children were released in Zambia (3 varieties) and Nigeria (2 varieties) in 2012. In South America, Embrapa of Brazil (www.biofort.com.br) has released a maize variety that contains similar levels of provitamin A. Varieties that can provide 50 percent of the EAR are in development.

African food processing and cooking methods result in provitamin A losses below 25 percent (Li *et al.* 2007). Storage stability studies with the varieties released in 2012 are not yet complete; a previous study of different varieties showed 25-60 percent decay of provitamin A after drying and four months of dark storage at 25° C (Burt *et al.* 2010). Bioavailability (the conversion rate of beta carotene to retinol) was originally assumed to be 12 to 1, but nutrition studies have found more efficient bioconversion rates of 3 to 1 and 6.5 to 1 (Muzhingi *et al.* 2011; Li *et al.* 2010). Nutritional efficacy studies in Zambia have concluded and results are expected to be published in 2014.

Cassava

Biofortified cassava is being developed for Nigeria and the Democratic Republic of the Congo (DRC). The initial breeding target was set at 15 ppm provitamin A. Screening research identified source germplasm from cassava populations in South America, and IITA and the International Center for Tropical Agriculture (CIAT) improved these varieties to be suitable to the African environment and resistant to cassava mosaic disease. Three varieties with sufficient provitamin A to provide 25 percent of the EAR for women and preschool children were released in Nigeria in 2011. Screening identified a variety with a similar level of provitamin A that was released in the DRC in 2008; it is now being disseminated to farmers. In South America, 3 varieties with up to 9 ppm provitamin A have been developed by Embrapa and released in Brazil.

As with maize, the bioavailability of provitamin A is much better than assumed; conversion of beta-carotene to retinol has been measured at 3.8 to 1 and 4.3 to 1, with and without added oil, respectively (Liu *et al.* 2010; LaFrano *et al.* 2012). Existing literature on the stability of provitamin A in cassava through traditional cooking processes highlights high losses (65-80 percent) associated with the most common form of cassava consumption in Nigeria, gari (Chavez *et al.* 2007; Thakkar *et al.* 2009). Given that retention is in part determined by cassava variety and great variability in processing methods exists at the household level, a study of retention during gari production and storage with recently released varieties was commissioned to confirm the tentative revision of the breeding target to 10 ppm. Results indicated an average retention of 40 percent of beta-carotene in freshly made gari, with further degradation during storage. According to the results, 50% of the vitamin A EAR can be supplied by gari that has been stored for up to 15 days for a child 3-5 years of age or up to 45 days for a woman of childbearing age. A nutritional efficacy trial is underway, with results expected in 2014.

Rice

In many Asian countries, rice provides up to 80 percent of the energy intake of the poor. High-zinc rice varieties for Bangladesh and India are developed by the International Rice Research Institute (IRRI) and the Bangladesh Rice Research Institute (BRRI). The initial breeding target has been revised to 28 ppm zinc in polished rice, an increment of 12 ppm above the baseline zinc concentration of commercially available rice. High-yielding varieties with more than 75 percent of the target are in official registration trials in India; the first high-zinc rice variety was released in Bangladesh in 2013. A high-zinc rice variety was identified in Brazil and released in 2012 by Embrapa, and a high-iron rice variety was released in China in 2011; research to incorporate the high-zinc trait into this Chinese line continues.

Retention studies showed that the zinc content of rice is not significantly reduced by parboiling and less so by milling relative to iron, as zinc is distributed more homogenously throughout the brown rice grain (Resurreccion *et al.* 1979; Liang *et al.* 2008). Controlled studies conducted by the Bangladesh Rice Research Institute quantified the loss of zinc from rice during milling and washing before cooking. Approximately 10 percent of the zinc in the milled grain was lost during washing prior to cooking (Juliano 1985). When rice is boiled in an excessive volume of water, which is discarded prior to serving, another 10-14 percent of the zinc may be lost (Dipti 2012). There is neither bioavailability nor efficacy evidence thus far for zinc-biofortified rice. Demonstrating efficacy of a food-based intervention to improve zinc intakes is challenging due to poor sensitivity of serum zinc concentration in response to relatively low amounts of additional zinc intake. More sensitive biochemical indicators of zinc status are needed, and more research is required to assess the impact of zinc interventions on human health.

Wheat

The development of high-zinc wheat for India and Pakistan is led by CIMMYT. The initial breeding target for whole wheat was revised to 37 ppm zinc, an increment of 12 ppm above the baseline zinc concentration. It is expected that adoption of high-zinc wheat will be driven by its improved agronomic properties compared to current popular varieties, and breeding has focused on both zinc content and resistance to new strains of yellow and stem rust. Multilocation trials are underway in both India and

Pakistan; commercialization of varieties with 75 percent of the zinc target level will begin in India in 2013. A wheat variety with zinc concentration of 44 ppm, well above the target level, was released in China in 2011 (www.harvestplus-china.org).

In general, wheat mineral losses are directly proportional to the duration and intensity of milling, but bioavailability increases due to simultaneous phytate reduction. The Punjab Agricultural University is assessing iron and zinc losses associated with traditional milling and cooking methods. An absorption study among women in Mexico showed that total absorbed zinc was significantly greater from the biofortified variety of wheat as compared with non-biofortified wheat (Rosado *et al.* 2009). Additional zinc absorption and efficacy research in 2013 will validate this result for genotype-specific variations in phytate concentration, as phytates have an inhibitory effect on iron absorption.

Pearl Millet

Pearl millet is a regionally important staple in the Indian states of Maharashtra, Rajasthan, Gujarat, and Uttar Pradesh, the target area for biofortified pearl millet. The breeding target was set at 77 ppm iron, an increment of 30 ppm above the baseline. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) carries out the pearl millet breeding research in collaboration with NARES and the private sector. The popular open pollinated variety (OPV) ICTP 8203 was improved to create the first biofortified variety, called ICTP 8203-Fe, which contains 100 percent of the iron target. ICTP 8203-Fe was commercialized in 2012 and officially released in 2013. Hybrid varieties with up to 100 percent of the iron target are in the development pipeline.

Human bioavailability of pearl millet iron is low (Guiro *et al.* 1991). A recent study comparing mineral absorption between a biofortified and control pearl millet variety in children 2-3 years of age in India found a significant difference in total iron absorbed in favor of the biofortified variety (Kodkany *et al.* 2013). Additionally, the iron absorption from ICTP 8203-Fe pearl millet when consumed as porridge was higher (7 percent) than initially assumed for setting breeding target levels (5 percent). A subsequent study validated these bioavailability results in women of child bearing age in Benin (Cercamondi *et al.* 2013).

Beans

The target countries for high-iron bean are Rwanda and DRC, and CIAT and the Rwandan Agricultural Board (RAB) lead the breeding process to reach the initial breeding target of 94 ppm, 44 ppm above the baseline. Several "fast track" lines with more than 60 percent of the target level of iron are in delivery. Five varieties of biofortified beans with higher iron levels were released in Rwanda in June 2012. The breeding pipeline includes both large-seeded bush lines and mid-altitude adapted climbing beans. In South America, three varieties with 80 ppm iron have been developed by AgroSalud and released in Brazil.

Background nutrition research suggests that bean consumption in Rwanda is about 10-20 percent lower than assumed when setting the target, a finding that is currently being validated. Apparent iron retention after cooking is close to 100 percent, as beans are not presoaked before cooking and none of the water used in cooking is discarded (USDA 2007). In general, single meal studies show iron bioavailability to be low (3-5 percent); there is some evidence now that these test meal studies may underestimate bioavailability over longer periods of time, as there is adaptation to high levels of polyphenols (Petry *et al*. 2012). Further research testing the hypothesis that human iron absorption adapts to high levels of dietary phytate is underway in Rwanda and the United States.

Lentil, Cowpea, Banana, Sorghum and Potato

Additional crops are being conventionally bred for higher micronutrient levels, as detailed below. In general, target levels as a percentage of EAR have not been determined, and more research is necessary to provide nutrition evidence for the efficacy of biofortification in these crops.

The International Center for Agricultural Research in the Dry Areas (ICARDA) leads research seeking to biofortify lentils with higher levels of iron and zinc. Multilocation testing began in 2009, and varieties have been tested in Bangladesh, Ethiopia, India, Nepal, and Syria. In Bangladesh, mineral-dense varieties identified in early screening are already promoted for wide-scale cultivation. In Nepal, a candidate variety (ILL 7723) was released in 2012.

Cowpea research is led by G.B. Pant University of Agriculture and Technology, Pantnagar, India. Two early-maturing high-iron and zinc cowpea varieties, Pant Lobia-1 and Pant Lobia-2 were released by the Uttarakhand Government in 2008 and 2010, respectively. These varieties have now entered the national seed multiplication system and seed is available to farmers. Brazil also released three varieties of high-iron cowpeas, developed by Embrapa, in 2008 and 2009.

Banana/plantain varieties providing up to 20 ppm provitamin A have been identified through germplasm screening in Nigeria, Ivory Coast, Cameroon, Burundi, and DRC by IITA and Bioversity. In Nigeria, planting materials have been disseminated. Planting materials of several varieties are currently being multiplied for dissemination to farmers.

Zinc- and iron-dense sorghum hybrids developed at ICRISAT will enter multilocation testing and on-farm adaptation trials in India in 2013. If competitive mineral-dense hybrids emerge, commercialization can be projected for 2015.

High-iron potato clones have been developed at CIP. After production of virus-free in-vitro plantlets, these clones are now being introduced to Rwanda and Ethiopia for testing for local adaptation.

Country Programs

Country programs in Brazil, China, and India work to biofortify a wide array of staple food crops. This "food basket approach", favored particularly in Brazil, is useful for addressing micronutrient deficiencies in populations that consume smaller amounts of several staple crops, rather than deriving most of their nutrition from a single staple crop.

BIOFORT Brasil is coordinated through the Brazilian Agricultural Research Corporation (Embrapa) and is developing nutrient-rich varieties of eight crops: rice, sweet potato, bean, cowpea, cassava, maize, wheat, and pumpkin. Ten varieties have been released: three each of cassava (up to 9 ppm provitamin A); beans (up to 80 ppm iron and 50 ppm zinc); and cowpeas (up to 77 ppm iron and 53 ppm zinc); and one sweet potato variety (up to 115 ppm provitamin A). Furthermore, a provitamin A maize variety (up to 7.5 ppm) was released in 2012.

China's biofortification program began in 2005 and research focuses on increasing iron, zinc, and provitamin A contents in rice, maize, wheat, and sweet potato. Released varieties include: a wheat cultivar, "Zhongmai 175", with 44 ppm zinc and 38 ppm iron; a rice variety, "Zhongguangxiang", with 6.5 ppm iron; and a sweet potato variety, "Nanshu 0101", with 93 ppm provitamin A. Additional promising provitamin A-rich sweet potato and maize varieties are in multilocational trials.

The Indian Government's Department of Biotechnology (DBT) and the Indian Council of Agricultural Research (ICAR) have joined efforts to achieve high-quality research and accelerate the development of biofortified varieties in India. The India Biofortification Program, a long-term project of the DBT, focuses on rice, wheat, and maize. HarvestPlus is a collaborator in the development of these crops and also focuses on biofortified pearl millet and sorghum in collaboration with ICRISAT.

2.2 Transgenics

Transgenic biofortification is used when genetic variability for vitamin and mineral targets is too low to meet the desired target levels, or for crops that are very difficult to breed, such as banana.

Golden and High-Iron Rice

Golden Rice was first developed at the Swiss Federal Institute of Technology, and research was furthered by Syngenta as part of their then-commercial pipeline. Transgenic events with higher levels of provitamin A, up to 37 ppm in a U.S. variety (the GR2 events), were produced and were then donated for use by the Golden Rice Network when Syngenta decided not to pursue the trait as a commercial product (Al-Babili and Beyer 2005). Research on Golden Rice is currently led by IRRI. Starting in 2006, the GR2 events were backcrossed into varieties for the Philippines, Indonesia, India, and Bangladesh (Beyer 2010). Field-testing is currently ongoing.

Bioavailability testing has confirmed that golden rice is an effective source of vitamin A in humans, with an estimated conversion rate of beta carotene to retinol of 3.8 to 1 (Tang *et al.* 2009). Golden rice will be required to pass biosafety tests prior to release; the data for this safety assessment are expected to be submitted to Philippines's regulators in 2013 and in Bangladesh after 2015. An efficacy trial is planned in the Philippines after biosafety approval is granted.

Additionally, a transgenic high-iron rice variety has been developed by the University of Melbourne and IRRI that contains 14 ppm iron in the white rice grain and translocates iron to accumulate in the endosperm, where it is unlikely to be bound by phytic acid and therefore likely to be bioavailable (Johnson *et al.* 2011). Bioavailability trials are expected to begin next year, and release is projected for about 2022 in Bangladesh and India.

BioCassava Plus

The BioCassava Plus (BC+) program genetically engineers cassava with increased levels of iron and provitamin A. Additional traits addressed by BC+ include increased shelf life, reduced cyanide levels, and improved disease resistance. The first field trials for a provitamin A biofortified cassava began in 2009, followed by trials for high-iron cassava (Sayre *et al.* 2011). Delivery of the biofortified crops is expected in 2017. Retention and bioavailability of transgenic cassava are similar to the findings of HarvestPlus on conventional biofortification research indicated above (Failla *et al.* 2012).

Banana Biofortification

Queensland University of Technology and the National Agricultural Research Organization of Uganda are developing transgenic provitamin A and iron bananas for Uganda. Bananas with up to 20 ppm provitamin A have been developed and trials have commenced in Uganda (Namanya 2011). Provitamin A bananas are expected to be released in 2019. A human bioavailability study using transgenic provitamin A banana will begin in early 2013. High-iron bananas are not yet ready for use in human trials.

African Biofortified Sorghum

Africa Harvest and Pioneer lead a consortium of institutions genetically modifying sorghum. Transgenic sorghum has elevated levels of provitamin A (up to 21 ppm), reduced phytate (35-80 percent), and an improved protein profile. Transgenic plants are currently in greenhouse trials; release is expected by 2018.

Sorghum has lower relative bioaccessibility in transgenic varieties, but the higher levels of carotenoids in the grain increased the overall levels of accessible total and provitamin A carotenoids. Additional bioavailability studies have shown increased zinc absorption of 30-40 percent and increased iron absorption of 20-30 percent when phytate levels are reduced.

3. Delivery and commercialization experiences

Two factors influence the design of a delivery strategy: whether the biofortified trait is visible or invisible, and the availability of good infrastructure (including the presence of well-developed seed sectors and seed markets) for dissemination. Provitamin A crops are visibly different, while crops biofortified with iron or zinc are visually indistinguishable from their non-biofortified counterparts. Figure 3 offers a framework that helps to inform dissemination strategies.

From 2007 to 2009, HarvestPlus and its various NGO partners distributed OSP to more than 24,000 households in Uganda and Mozambique (HarvestPlus 2010; Hotz *et al.* 2012b). The pilot delivery project was new to Uganda but built on two previous CIP projects in Mozambique, *Towards Sustainable Nutrition Improvement* and *Eat Orange*. Because there were no markets for sweet potato vines in Uganda and Mozambique, planting materials were delivered through NGO partners. An operations research component monitored implementation activities, while a parallel impact evaluation team carried out a randomized control study. The impact evaluation component tested two delivery methods: an intensive method that included two years of planting material delivery and training, and a less in-

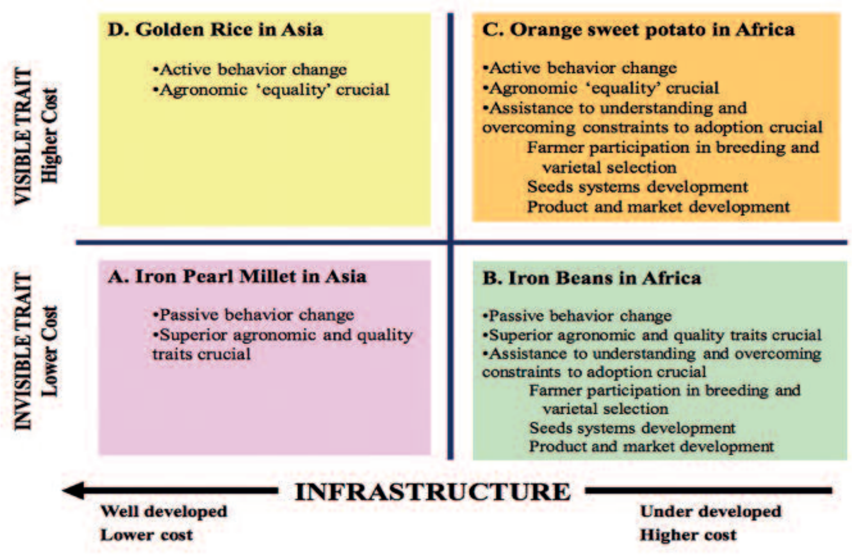

Figure 3. Delivery Framework.

tensive method that included only one year of planting material delivery and training. The less intensive model was shown to be as effective as the more intensive one; in both countries the project led to increases in OSP adoption and consumption by farm households. As a result, vitamin A intakes as much as doubled for both children and women, the primary target groups for this intervention.

Dissemination can also utilize the private sector. In India, HarvestPlus is taking advantage of the existing well-functioning seed sector for pearl millet, and has partnered with a private seed company, Nirmal Seeds Ltd., to market and deliver ICTP 8203-Fe. By partnering with a leading commercial entity in pearl millet seed sales in the target state of Maharashtra, HarvestPlus is increasing demand in an existing market and building a sustainable strategy for future delivery. Because the high iron trait is an invisible one, demand for the ICTP 8203-Fe will be driven by its superior yield performance compared to the earlier version. The marketing of invisible traits is informed by the delivery experience in Rwanda, where over 500,000 packs of high-iron beans have been distributed to 150,000 households since 2011.

4. Effectiveness of biofortification

The primary evidence for the effectiveness of biofortification comes from orange sweet potato (OSP). Effectiveness was assessed through a randomized control trial in both countries. The pilot delivery project described above resulted in a 68 percent increase in the probability of OSP adoption in Mozambique and a 61 percent increase in Uganda (Hotz *et al.* 2012a; Hotz *et al.* 2012b). OSP adoption resulted in substantial substitution of other sweet potato varieties in terms of area under cultivation; the project increased the share of OSP in total sweet potato areas by 59 percent in Mozambique and 44 percent in Uganda. Compared to intakes at baseline, vitamin A intake doubled for all three age/gender groups by project end in Mozambique, and in Uganda increased by two-thirds for younger and older children and nearly doubled for women. For the age group of greatest concern, children aged 6-35 months, OSP contributed 74 percent of the total vitamin A intake in Mozambique and 52 percent in Uganda (see Figure 4). In Uganda, the high prevalence of inadequate vitamin A intake among a subset of children 12-35 months, who were no longer breastfeeding, fell from nearly 50 percent to only 12 percent as a result of the

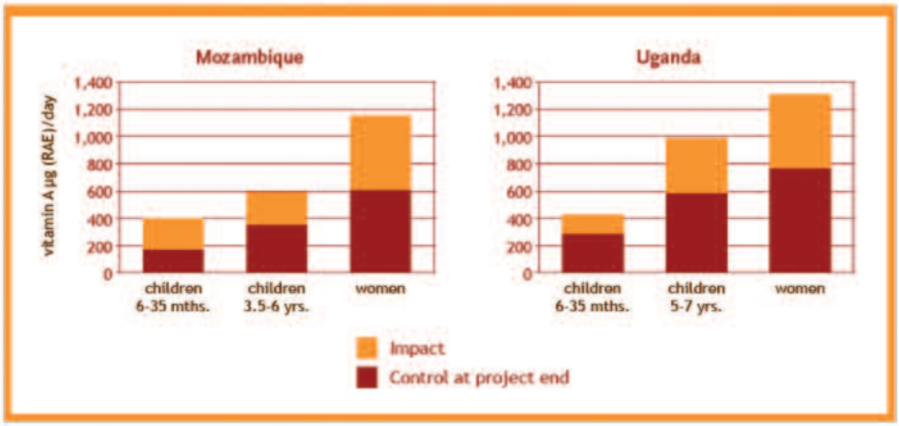

Figure 4. Impact of REU Intervention on mean vitamin A intakes (µg Retinol Activity Equivalents (RAE)/day), Mozambique and Uganda by age group.

Notes: *Estimates are mean vitamin A intakes at project end (2009) in both countries. Mean vitamin A intakes at baseline were not significantly different between project and control households within each age group. For younger children in both countries, separate groups of children were assessed at the beginning and end of the project. For older children and women, the same group was followed over time. Retinol is the active form of vitamin A found in the body. Beta carotene is converted to retinol by the body and the amount of retinol derived from beta carotene is expressed as retinol activity equivalents (RAE).*

project. Researchers were also able to measure a modest yet significant impact of eating OSP on the amount of vitamin A in the blood among children 5-7 years who had lower levels of vitamin A at the start of the project. At project end, researchers also found that women who got more vitamin A from OSP had a lower likelihood of having marginal vitamin A deficiency (Hotz *et al.* 2102a).

Disability Adjusted Life Years (DALYs) are a commonly used metric for measuring the cost-effectiveness of health interventions. For example, in Uganda, calculations suggest that the intervention cost US$15-US$20 per DALY saved, which by World Bank standards is considered highly cost effective (World Bank 1993; HarvestPlus 2010). An ex ante cost-effectiveness study by HarvestPlus estimated that consumption of OSP could eliminate between 38 and 64 percent of the disability-adjusted life years (DALYs) burden of vitamin A deficiency in Uganda (Meenakshi *et al.* 2010).

5. Promise and potential barriers for biofortified crops

Consumer Acceptance and Farmer Adoption

In the pilot delivery programs in Mozambique and Uganda, when beneficiaries were provided information (i) that consumption of orange sweet potato could protect their children from the consequences of vitamin A deficiency and (ii) that orange sweet potato varieties were just as high yielding as white varieties, these households produced and consumed orange varieties – commensurately lowering their production and consumption of white sweet potato.

Rural consumers want nutritious food and are willing to pay a price premium for it, indicating favorable valuation of and demand for staple foods with nutritional benefits. When given the same information as above under experimental conditions, "willingness-to-pay" studies for orange sweet potato, orange maize, and yellow cassava showed that consumers liked the sensory characteristics of the biofortified crops and will pay a higher price for high provitamin A varieties than for white varieties (Chowdhury *et al.* 2011; Meenakshi *et al.* 2012; Oparinde *et al.* 2012).

Consumer acceptance of crops biofortified with invisible nutrition traits (such as high-iron pearl millet) and the impact of branding/certification was also tested. In the case of high-iron pearl millet, it was found that even in the absence of nutrition information, the high-iron variety was preferred to the local variety (Banerji *et al.* 2012).

Varietal adoption studies inform efficient, effective, and targeted crop development and delivery strategies by providing information about current crop production practices, including currently popular varieties of crops, preferred agronomic and consumption traits, sources of information about new varieties, and sources of seed. Following the delivery of biofortified crops, HarvestPlus will study farmer adoption and diffusion, collecting feedback from farmers on agronomic and consumption characteristics and estimating the number of direct and indirect adopters.

Crop Performance

High mineral and vitamin density can be bred into the edible portions of staple foods while maintaining high yields, resistance to pests and diseases, and other desirable agronomic traits.

Without desirable agronomic traits, farmers will not adopt biofortified staple crops; each variety released must be at least competitive with what is available in the market.

Transgenics

Several transgenic biofortified crops are currently under development that also have novel agronomic traits (e.g. disease resistance).

While transgenic approaches may reduce time-to-market, resistance to accepting transgenic crops remains high, particularly in Africa.

6. Conclusions

Major gaps in knowledge with respect to biofortification exist: more efficacy trials and effectiveness studies are needed to confirm and augment the promising evidence thus far obtained. Scientists must further refine indicators of individual micronutrient status and better understand the importance of cross-nutrient synergies. Additional delivery and marketing research will improve the effectiveness of delivery and marketing strategies in ensuring maximum adoption and consumption of biofortified crops.

Breeding can be made more cost-effective using marker-assisted selection to breed high levels of several minerals and vitamins in a single variety, and transgenic methods may prove to be more effective in accomplishing this than conventional breeding. To mainstream biofortified traits, agricultural research centers must adopt breeding for nutrient density as a core activity, investing in breeding pipelines at NARES. National varietal release committees should be encouraged to set minimum standards for nutrient densities in the crops that are released; currently only agronomic standards are considered.

Furthermore, biofortification is complementary to existing interventions, but the best mix of biofortification, supplementation, fortification, and dietary diversity must be considered for each target country, and the coordination of these programs must be improved.

Looking forward, a range of institutions must be convinced to endorse the biofortification strategy. Key actors in expanding dissemination globally and ensuring sustainability include the UN and related agencies, international and regional programs such as Scaling Up Nutrition (SUN) and the Comprehensive African Agricultural Development Program (CAADP), international NGOs, seed and food companies, and donor agencies. Only through broadening the biofortification coalition will long-term support for breeding and dissemination of biofortified crops be realized.

Concluding remarks

It is obvious, but sometimes forgotten, that agriculture and food systems will always provide most of the nutrients and compounds that humans require to sustain healthy and productive lives. Agriculture has several roles to play (e.g. also to provide employment and income), but this is surely its most important function. It is clear that agriculture has not performed this function well in developing countries – in the face of rapidly growing populations and land, environmental, human capacity, and institutional constraints. Biofortification is yet to be fully scaled up in a single country but much evidence and experience has been assembled to support its eventual effectiveness. Although the knowledge gaps and tasks ahead may seem daunting, investment in biofortification is a cost-effective approach to ensure a more nourishing future.

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