HarvestPlus
Better Crops • Better Nutrition

Biofortification Progress Briefs

August 2014

PHOTO BY N. PALMER (CIAT)

Introduction to Biofortification Progress Briefs

August 2014

The following briefs were solicited by HarvestPlus for the Second Global Conference on Biofortification, "Getting Nutritious Foods to People," which took place in Kigali, Rwanda from March 31 to April 2, 2014. The conference, an interactive global consultation attended by more than 300 leaders in agriculture, food, nutrition, and health, was officially hosted by the Government of the Republic of Rwanda and organized by HarvestPlus. The conference culminated in a series of commitments to tackle hunger and micronutrient deficiency through nutrition-sensitive agriculture, captured in the Kigali Declaration on Biofortified Nutritious Foods. To learn more about the conference and its outcomes, please visit http://biofortconf.ifpri.info/.

The briefs were developed as background information for the conference and are intended to present existing evidence on biofortification, identify knowledge gaps, and stimulate discussion on how to leverage biofortification to improve nutrition and health. They are meant to be accessible to a variety of audiences, from researchers to practitioners working on the ground. Readers interested in learning more about these topics can follow the references to journal articles and working papers that underpin many of the briefs. The briefs have proven to be very popular, and will be further refined in the coming months and publically launched on the HarvestPlus website.

We hope that you will find these briefs useful and that they will inspire a dialogue among you and your partners as you seek new pathways to improve nutrition and public health.

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Director, HarvestPlus

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Parminder Virk (CIAT-HarvestPlus)

Table 1. Summary of Zinc Rice

Target Micronutrient		Zinc. Secondary trait: iron	
Target Countries		Bangladesh, India	
Baseline *(parts per million, ppm)*		16 ppm	
Target Increment		+12 ppm (increased from original target of 8 ppm)	
Target Level in crop		28 ppm	
Nutrition Factors		**Original Assumption**	**Measured/ Revised**[1]
Rice Consumption *grams/day (dry weight)*	Women	400 g/d	422 g/d
	Children	200 g/d	169 g/d
Zinc Retention (%)		90%	90%
Zinc Absorption (%)		25%	20%
Absorbed incremental zinc as % of EAR		40%	36%
Releases			
1st Wave	+6–8 ppm (33–66% target increment)	Release: Bangladesh, 2013; India, 2015	
2nd Wave	+8–12 ppm (50–75% target increment)	Planned release: 2015	
3rd Wave	>+12 ppm (>100% target)	Planned release: 2017	

[1] Bangladesh

Breeding to Date

Initial screening of 939 rice genotypes by the International Rice Research Institute (IRRI) found concentrations of 15–58 ppm zinc (and 7.5–24 ppm iron) in unpolished rice grain (1). Because zinc is spread throughout the endosperm, estimates of zinc in unmilled rice are reliable indicators of zinc in milled rice; this is not the case for iron, as much of the iron in the aleurone layer is lost during milling (2). Genotype-by-environment (GxE) testing was used to evaluate the most promising germplasm and verify that mineral accumulation was stable across sites and generations. Positive correlation between iron and zinc allows for simultaneous improvement of both minerals. Research efforts continue to identify quantitative trait loci (QTLs) associated with grain zinc content and to better understand zinc uptake, transport, and remobilization into the grain (3). In HarvestPlus Phase II (Development, 2009–2013), the validity and precision of various mineral analysis methods were studied, and inductively-coupled plasma (ICP) approaches were developed for zinc and iron in rice. X-ray fluorescence (XRF) spectrometry calibrations and standards were developed for high-throughput screening (4).

Breeding programs at IRRI and the Bangladesh Rice Research Institute (BRRI) have assumed full operational scale for breeding of zinc rice. A full breeding pipeline consists of germplasm in early- to late-development stages and elite line final products. At the Indian National Agricultural Research System (NARS), a breeding pipeline is being developed, focusing on varieties for the *kharif* season. Due to geographical proximity and agroecological similarity, adaptation of Bangladesh high-zinc rice leads in eastern India can be expected. Rice hybrids and respective parental inbreds were assessed for zinc; however, zinc hybrid breeding is not currently planned. Mainstreaming of the zinc trait at IRRI and BRRI is estimated at 25 percent of the rice breeding effort.

HarvestPlus has screened more than 7,500 rice lines and identified several high-zinc genotypes among unadapted sources for use as donor parents in the zinc breeding program. The aim is to produce competitive zinc-dense varieties with high yield, abiotic and biotic stress tolerance, and end-use quality attributes required for adoption. Breeding effectiveness at BRRI is accelerated by introduction of more than 3,000 early and advanced high-zinc lines from IRRI each year, selected based on grain yield and grain zinc for GxE testing under local conditions.

In Bangladesh, the first zinc rice *Aman* (rainfed) season variety, “BRRI dhan 62,” was released in 2013. It has 20 ppm zinc and is the shortest duration *Aman* rice variety ever released. At least one B*oro* (irrigated) season zinc rice variety with 22–24 ppm is expected to be released in 2014. In India, the first varieties are expected to be commercialized in 2015.

Future Releases

The major focus is on developing higher yielding, zinc pure-line varieties for both *Boro* and *Aman* seasons with stable yield performance across different agroecological zones, including cold tolerance at seedling stage and heat tolerance at post flowering stage in the main *Boro* season.

Resistance to diseases such as bacterial leaf blight and blast is an integral part of the breeding program. User-preferred quality traits such as high amylose, long and slender grains, and short duration are also combined with competitive yield.

Capacity Building

XRF machines have been installed at BRRI and Indian NARS. Since 2011, the mineral analysis of all rice samples produced is done by XRF in-country, resulting in reduced analysis costs and time savings.

Regional Testing

Competitive high-zinc rice varieties and elite lines are tested regionally through IRRI's International Network for Genetic Evaluation of Rice (INGER), a germplasm dissemination and evaluation tool. Agronomic and grain zinc data from large-scale GxE testing at multiple sites allow for higher effectiveness in targeted breeding for yield and zinc stability based on adaptive pattern, as well as the identification of fast-track candidates and parents for breeding.

Starting in 2013, a zinc rice nursery was distributed to collaborators across India through the All India Coordinated Rice Improvement Project and tested under various production conditions. By substituting temporal-by-spatial environmental variation in large-scale regional GxE testing, testing steps can be eliminated and time-to-market shortened by 1–2 years. Zinc rice varieties are also being tested in Bolivia, Brazil, Colombia, Indonesia, Nicaragua, Panama, and the Philippines.

Highlights

- BRRI dhan 62, a modern, short duration, and medium slender-grained zinc rice variety for *Aman* season, was released ahead of schedule in 2013.
- In-country capacity for mineral analysis has been established in Bangladesh and India.

Challenges

- Grain yield and mineral density are affected by environmental and GxE effects, but interactions are not well understood.
- Limited genetic variation for zinc and iron in rice constrains increases that can be realized through conventional biofortification.

Table 2. First-Wave Varieties for Bangladesh

Variety Name	**Zinc Content[1]**		**Grain Yield (% over check)**		**Growth Duration[2] (days)**
	ppm	*Zinc Increase*	*t/ha (6 sites[1])*	*t/ha (11 sites[2])*	
***Aman* Variety – Released in 2013**					
BRRI dhan 62	19.6	+7.9 ppm	4.2		100
***Boro* Varieties – At least one expected to be released in 2014**					
BR7840-54-3-1	24.7	+7.9 ppm	5.63 (100%)	6.32 (102%)	147
BR7840-54-2-5-1	22.7	+5.9 ppm	5.87 (104%)	6.31 (102%)	148
BR7840-54-1-2-5	23.6	+6.8 ppm	5.81 (103%)	6.42 (103%)	140
BRRI dhan 28 (Control)	*16.8*	-	*5.62 (100%)*	*6.21 (100%)*	*144*

[1] Mean across 6 sites of PVS trials during 2011/12 *Boro* season (Zn measured by XRF)

[2] Mean across 11 sites of ALART trials during 2011/12 *Boro* season.

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PROGRESS BRIEF #2

CROP DEVELOPMENT

Zinc Wheat

Parminder Virk (CIAT-HarvestPlus) & Velu Govindan (CIMMYT)

Table 1. Summary of Zinc Wheat

Target Micronutrient		Zinc. Secondary trait: iron	
Target Countries		India, Pakistan	
Baseline *(parts per million, ppm)*		25 ppm	
Target Increment		+12 ppm (increased from original target of 8 ppm)	
Target Level in Crop		37 ppm	
Nutrition Factors		**Original Assumption**	**Measured/ Revised**[1]
Wheat Consumption, *grams/day (dry weight)*	Women	400 g/d	208g/d
	Children	200 g/d	72 g/d
Zinc Retention (%)		90%	90%
Zinc Absorption (%)		25%	15%
Absorbed Incremental Zinc as % of EAR		40%	20%
Releases			
1st Wave	+4–8 ppm (33–66% target increment)	Commercialized: India, 2014 Planned release: Pakistan, 2015	
2nd Wave	+8–12 ppm (66–100% target increment)	Planned release: 2016	
3rd Wave	>+12 ppm (>100% target)	Planned release 2018	

[1] Punjab, India

Breeding to Date

During HarvestPlus Phase I (Discovery, 2003–2008), initial screening of more than 3,000 germplasm accessions by the International Maize and Wheat Improvement Center (CIMMYT) found ranges of 20–115 ppm zinc (and 23–88 ppm iron) in wheat, with the highest levels found in landraces; high-zinc genotypes were selected to initiate crosses (1). Multi-environment testing was conducted to evaluate the most promising germplasm and verify that mineral accumulation was stable across sites and generations. While variances were associated with environmental effects, high heritabilities were observed for zinc and iron concentrations across environments (2). Research efforts continue to identify quantitative trait loci (QTLs) associated with grain zinc content and examine how to increase zinc loading in the grain (3). In Phase II (Development, 2009–2013), the validity and precision of various mineral analysis methods were studied, and X-ray fluorescence (XRF) spectrometry calibrations and standards were developed for high-throughput screening (4).

Breeding programs at CIMMYT, the National Agricultural Research System (NARS), and agricultural universities in India and Pakistan have assumed full operational scale. Breeding efforts focus on transferring the zinc trait from diverse sources into locally adapted, agronomically competitive germplasm, considering consumer preferred end-use quality attributes. Resistance to the yellow rust Yr27 was mandatory in germplasm developed under HarvestPlus; resistance to the stem rust race Ug99 was built into zinc wheat as sources became available. Mainstreaming of the zinc trait at CIMMYT, as a percentage of the global wheat breeding effort, and at Indian and Pakistani partner NARS is estimated at 25–30 percent.

Breeding effectiveness in developing zinc wheat for India and Pakistan was optimized by the selection of 100–150 promising advanced lines at CIMMYT each year, based on grain yield and grain zinc, for testing in genotype-by-environment (GxE) trials for agronomic attributes and grain zinc at 10–15 sites in India and at 5 sites in similar agroecologies in Mexico and Pakistan (HarvestPlus South Asia Screening Nursery). The best 40–50 emerging leads are then yield-tested in multi-location yield trials (HarvestPlus South Asia Yield Trial) at more than 20 sites in India and Pakistan. Partners engaged in both countries are the public sector NARS and several private seed companies.

In India, six first-wave leads, selected on the basis of multi-location performance and zinc data, were commercialized for test marketing in 2013 and will be more widely commercialized in 2014. In Pakistan, three candidate varieties were submitted to official registration trials in 2012; at least one first-wave variety is expected to be released in 2015.

Future Releases

Lines being evaluated for second-wave commercialization demonstrate 75–100 percent of the zinc target. Agronomic and zinc data from multiple sites will be used to identify the best performers, and intensive on-farm, mini-kit testing of several candidate lines will determine which leads will be commercialized based on performance and farmers' preference. Commercialization of second-wave varieties is anticipated in 2016.

Capacity building

XRF machines have been installed at three NARS partners in India (DWR, PAU, and BHU) and one in Pakistan (PARC). Since 2012, the mineral analysis of all wheat samples produced is done by XRF in-country, resulting in reduced analysis costs and time savings.

Regional Testing

Starting in 2014, a zinc wheat nursery will be distributed to collaborators across India through the All India Coordinated Wheat Trial system and tested under various production conditions, including different planting dates. By substituting temporal-by-spatial environmental variation in large-scale regional GxE testing, testing steps can be eliminated and time-to-market shortened by 1–2 years.

HarvestPlus is also engaging seed companies in GxE testing and commercialization of zinc wheat, and supporting companies in developing their own zinc varieties for commercialization by analyzing seed companies' advanced wheat lines for zinc free of charge. Zinc wheat varieties are also being tested in Bangladesh, Brazil, China, Ethiopia, and Nepal.

Highlights

- The first zinc wheat lines were commercialized for test marketing in India.
- In-country capacity for mineral analysis has been established in India and Pakistan.

Challenges

- Grain yield and mineral density are affected by environmental and GxE effects, but these interactions are not well understood.

Table 2. First-Wave Varieties for India and Pakistan[1]

Variety Name	Zinc Increase	Yield	Comments on Agronomic Properties
India – Commercialized in 2014			
BHU1	+4–10 ppm	5.0 t/ha	84 days to heading and 126 days to maturity
BHU3	+6–8 ppm	4.4 t/ha	83 days to heading and 125 days to maturity
BHU5	+4–5 ppm	3.3 t/ha	86 days to heading and 128 days to maturity
BHU6	+4–9 ppm	3.4 t/ha	78 days to heading and 119 days to maturity
BHU17	+6–10 ppm	4.1 t/ha	81 days to heading and 122 days to maturity
BHU18	+6–9 ppm	3.9 t/ha	87 days to heading and 131 days to maturity
Pakistan – Planned Release in 2015			
NR-419	+7–9 ppm	4.5 t/ha	93 days to heading and 130 days to maturity
NR-420	+7 ppm	3.4 t/ha	86 days to heading and 128 days to maturity
NR-421	+14 ppm	3.6 t/ha	78 days to heading and 119 days to maturity

[1] First wave: 50–66% target increment

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Iron Common Bean (*Phaseolus vulgaris*)

Steve Beebe (CIAT) & Meike Andersson (CIAT-HarvestPlus)

Table 1. Summary of Iron Bean

Target Micronutrient		Iron. Secondary trait: zinc	
Target Countries		Rwanda, Democratic Republic of Congo, Uganda	
Baseline *(parts per million, ppm)*		50 ppm	
Target Increment		+44 ppm	
Target Level in Crop		94 ppm	
Nutrition Factors		**Original Assumption**	**Measured/Revised**[1]
Bean Consumption, *grams/day (dry weight)*	Women	200 g/d	123 g/d
	Children	100 g/d	65 g/d
Iron Retention (%)		90%	98%
Iron Absorption (%)		5%	7%
Absorbed Iron as % of EAR		60%	60%
Releases			
1st Wave	+20-27 ppm (50–60% target increment)	Released: Rwanda, 2010; DRC, 2011	
2nd Wave	+30-40 ppm (80–90% target increment)	Released: Rwanda, 2012; DRC, 2013	
3rd Wave	>+44 ppm (>100% target)	Planned: 2015	

1 Rwanda

Breeding to Date

Common bean is the most common food legume in Latin America and eastern and southern Africa. It is cultivated as both bush and climbing growth habits. During an exploratory phase (1994–2002), initial screening of more than 1,000 bean germplasm accessions by the International Center for Tropical Agriculture (CIAT) found ranges of 30–110 ppm iron (and 25–60 ppm zinc) in common beans. During HarvestPlus Phase I (Discovery, 2003–2008), high-iron genotypes were used to initiate crosses to combine the high-mineral trait with acceptable grain types and agronomic characteristics (1). Genotype-by-environment (GxE) testing was used to verify that mineral accumulation was stable across sites and generations (2). In Phase II (Development, 2009–2013), a number of lines were identified that expressed more than 80 percent of the target level. Inductively-coupled plasma (ICP) was identified as the gold standard for high-precision mineral analysis capable of detecting soil contamination (3,4), and X-ray fluorescence (XRF) spectrometry calibrations and standards were developed for high-throughput screening (article in preparation). Breeding programs in target countries Rwanda (Rwanda Agriculture Board—RAB) and the Democratic Republic of Congo (L'Institut National pour l'Etude et la Recherche Agronomique—INERA) have developed crosses locally and are assuming a greater portion of the selection work. A full breeding pipeline consists of both locally developed germplasm and CIAT introductions. Mainstreaming of the iron trait in breeding programs at both CIAT and RAB is estimated at 50 percent, and 30 percent for INERA. So far, nine varieties have been released in Rwanda and 10 in DRC. In Rwanda, four first-wave, fast-track varieties (2 bush, 2 climber) were released in 2010 and five second-wave climbing bean varieties in 2012. In DRC, five first-wave, fast-track varieties (3 bush, 2 climber) were identified for release and dissemination in 2011 and five second-wave varieties (3 bush, 2 climber) in 2013. Varieties with good yield and farmer-preferred end-use quality in major market classes are given in the table below.

Future Releases

Currently, about 100 climber and bush bean lines are in advanced line validation trials to identify agronomically competitive third-wave varieties; leads have 90–100 percent target increment and release is projected for 2015. Future breeding efforts will focus on developing higher yielding, robust, high-iron varieties for a wider range of agroecological zones covering a broad range of market classes (grain color and size, cooking time, and taste).

Capacity Building

In 2011, RAB's and INERA's analytical capacities were strengthened by installing and implementing X-ray fluorescence (XRF) machines for in-country mineral analysis of beans. The XRF at RAB serves as the mineral analytical hub for Africa; the second XRF installed in nearby Bukavu (DRC) serves as backup. To date, more than 4,000 bean samples from Rwanda, Uganda, Democratic Republic of Congo (DRC), and Burundi have been screened for iron and zinc.

Regional Testing

Since 2012, a 50-entry regional nursery comprising released varieties and elite high-iron breeding leads from different countries has been deployed each crop season for GxE testing in Rwanda, DRC, Uganda, Burundi, and Malawi. From 2014 onwards, testing will be expanded to Tanzania, Kenya, and South Africa. The regional nursery serves as a germplasm dissemination and testing tool. Agronomic and iron data from multiple sites per country allow high precision identification of fast-track candidates and parents for breeding, as well as higher effectiveness in targeted breeding for yield and iron stability based on adaptive pattern. Further, by substituting temporal-by-spatial environmental variation in large-scale regional GxE testing, testing steps can be eliminated and time-to-market shortened by one to two years. Iron bean varieties are also being tested in Guatemala, Honduras, and Nicaragua.

Highlights

- Competitive iron varieties nearing 90% of target increment and covering a wide range of market classes have been released in Rwanda and DRC.
- Many thousands of bean growers have been reached through intensive efforts at seed production and distribution.
- In-country capacity for mineral analysis has been established in Rwanda and DRC.
- A feeding trial with college-age women demonstrated positive health effects of iron beans (see nutrition section).

Challenges

- Plant breeding may focus on reducing uptake inhibitors, including phytate, which can inhibit iron absorption from beans. However, it is difficult to maintain acceptable yield while selecting for the reduced phytate trait.
- Efforts to improve yield will result in more productive varieties, and it will always be necessary to continue breeding to ensure that the better yield is accompanied by high iron.

Table 2. First- and Second-Wave Varieties Released in Rwanda and DRC

Variety Name	Release Year	Iron Content* (% target)	Altitude Range; Color; Disease Reaction
Rwanda - First wave (fast-track)			
RWR 2245 (Bush)	2010	+26 ppm (59%)	Low to mid altitude; color red mottled; AB, AC resistance; ALS, RR tolerance
RWR 2154 (Bush)	2010	+21 ppm (47%)	Low to mid altitude; color sugar; AB, AC resistance; ALS tolerance
MAC 44 (Climber)	2010	+28 ppm (64%)	Mid to high altitude; color red mottled; AC resistance; AB, ALS, RR tolerance
RWV 1129 (Climber)	2010	+27 ppm (61%)	Mid to high altitude; color salmon; AC, RR resistance; AB, ALS tolerance
Rwanda - Second wave			
RWV 3006 (Climber)	2012	+28 ppm (64%)	Mid to high altitude; color white; AB, AC, ALS resistance
RWV 3316 (Climber)	2012	+37 ppm (84%)	High altitude; color red; AC resistance; AB, ALS tolerance
RWV 3317 (Climber)	2012	+24 ppm (54%)	High altitude; color sugar; AC resistance; AB, ALS tolerance
MAC 42 (Climber)	2012	+41 ppm (94%)	High altitude; color sugar; AC resistance; AB, ALS tolerance
RWV 2887 (Climber)	2012	+35 ppm (80%)	High altitude; color dark red; AC resistance; AB, ALS tolerance
DRC - First wave (fast-track)			
COD MLB 001 (Bush)	2008	+14 ppm (32%)	Low to mid altitude; red mottled; AB, AC resistance; ALS, RR, drought tolerance
VCB 81013 (Climber)	2008	+19 ppm (43%)	Mid to high altitude; color white; AC, CBB, RR resistance; ALS tolerance
Hm 21-7 (Bush)	2008	+12 ppm (27%)	Low to mid altitude; red mottled; AB, AC, RR resistance; ALS, drought tolerance
RWR 2245 (Bush)	2011	+26 ppm (59%)	Low to mid altitude; color red mottled; AB, AC resistance; ALS, RR tolerance
COD MLV 059 (Climber)	2012	+34 ppm (77%)	Mid to high altitude; color red mottled; AC, CBB, RR resistance; ALS tolerance
DRC - Second wave			
PIGEON VERT (Bush)	2013	+30 ppm (68%)	Low to mid altitude; yellow; AC, BSM, CBB, RR resistance; LSF, drought tolerant
PVA 1438 (Bush)	2013	+29 ppm (66%)	Mid to high altitude; color red kidney; CBB, RR resistance
COD MLB 032 (Bush)	2013	+26 ppm (60%)	Mid to high altitude; color sugar; AB, AC resistance; ALS, RR, drought tolerance
CUARENTINO (Climber)	2013	+50 ppm (114%)	Mid to high altitude; color white; AC, CBB resistance; RR tolerance
NAIN DE KYONDO (Climber)	2013	+26 ppm (60%)	Mid to high altitude; color white; ALS, RR resistance; AB tolerance

*Average across four seasons, ICP & XRF data. Notes: AB: Ascochyta blight; AC: Anthracnose; ALS: Angular leaf spot; BCMV: Bean common mosaic virus; RR: Root rot

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Iron Pearl Millet

Kedar Rai (ICRISAT)

Table 1. Summary of Iron Pearl Millet

Target Micronutrient		Iron. Secondary trait: zinc	
Target Country		India. Secondary countries: West Africa	
Baseline *(parts per million, ppm)*		47 ppm	
Target Increment		+30 ppm	
Target Level in Crop		77 ppm	
Nutrition Factors		**Original Assumption**	**Measured/Revised**[1]
Pearl Millet Consumption, *grams/day (dry weight)*	Women	300 g/d	244 g/d
	Children	150 g/d	72 g/d
Iron Retention (%)		90%	95%
Iron Absorption (%)		5%	7-7.5%
Absorbed Incremental Iron as % of EAR		60%	60%
Releases			
1st Wave (OPV)	+6-8 ppm (92% target increment)	Commercialized: India, 2012. Released: 2013	
2nd Wave (Hybrid)	+8-12 ppm (88% target increment)	Commercialized: India, 2014	
3rd Wave (Hybrid)	>+30 ppm (>100% target)	Planned: 2016	

[1] Maharashtra, India

Breeding to Date

During HarvestPlus Phase I (Discovery, 2003–2008), initial screening of germplasm accessions by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) found ranges of 30–76 ppm iron (and 25–65 ppm zinc) in pearl millet; high-iron genotypes were selected to initiate crosses (1). High correlation between iron and zinc content indicated good prospects for simultaneous selection for the two micronutrients. Both micronutrients are largely under additive genetic control, implying that iron hybrids will require both parental lines to have high-iron density. Genotype-by-environment (GxE) testing was used to evaluate the most promising local germplasm and potential parents and verify that mineral accumulation was stable across sites and generations (2). In Phase II (Development, 2009–2013), breeding lines and germplasm with more than 90 ppm iron and more than 60 ppm zinc were validated. The validity and precision of various mineral analysis methods were studied, and near-infrared reflectance spectroscopy (NIRS) was calibrated for iron (2). X-ray fluorescence (XRF) spectrometry calibrations and standards were developed for high-throughput and cost-effective large-scale screening (3).

The breeding program at ICRISAT has assumed full operational scale. A full breeding pipeline initially included open-pollinated variety (OPV) development but now concentrates on hybrids and hybrid-parent development. Almost all identified iron sources are based on *iniadi* germplasm (early-maturing, large-seeded landrace materials from a geographic area adjoining Togo, Ghana, Burkina Faso, and Benin) or have a large proportion of *iniadi* germplasm in their parentage. The major focus of the breeding program is to develop higher yielding, high-iron hybrids with stable yield and iron performance for the different agroecological zones in India. Major traits include drought tolerance, resistance to downy mildew, and end-use quality traits. Research partners in India include five State Agricultural Universities and 15 seed companies. HarvestPlus engages State Agricultural Universities and seed companies in GxE testing of hybrids and inbred lines developed at ICRISAT. It also encourages them to develop their own high-iron hybrids for commercialization by analyzing seed company hybrids and inbred lines for iron free of charge. Mainstreaming of the iron trait at ICRISAT is estimated at 40–45 percent.

Photo: A.S. Rao (ICRISAT)

An iron OPV, ICTP 8203-Fe, was commercialized as Truthfully Labeled Seed (TLS) by Nirmal Seeds in the 2012 rainy season in Maharashtra and officially released for that state in 2013. Due to its high iron content and wide adaptation, ICTP 8203-Fe was notified as "Dhanashakti" in February 2014 for cultivation in all pearl millet-growing states of India.

Future Releases

Two agronomically competitive hybrids with up to 90 percent of the iron target increment, developed at ICRISAT, have been identified as leads for second-wave commercialization/release. These are being multiplied by two commercial seed companies, Nirmal Seeds and Shakti Vardhak, for commercialization as TLS in 2014.

Capacity Building

In 2010, ICRISAT's analytical capacity was strengthened by implementing XRF spectrometry for mineral analysis; a backup XRF was installed in 2012. To date, more than 45,000 pearl millet samples from ICRISAT's breeding program, the Indian National Agricultural Research System (NARS), and private sector collaborators have been assayed for iron and zinc.

Regional Testing

The improved variety ICTP 8203-Fe is expected to perform as well as ICTP 8203 in Namibia and Zimbabwe. Iron pearl millet is also being tested in Niger.

Highlights

- The first iron OPV, ICTP-8203-Fe, was commercialized in Maharashtra by Nirmal Seeds in 2012.
- Competitive iron hybrids approaching 90 percent of target increment will be commercialized in 2014.
- In-country capacity for mineral analysis has been established in India.

Challenges

- Seed companies dominate the seed market for pearl millet hybrids in India, and approximately 95 percent of the area under improved cultivars (OPVs and hybrids) in India is planted with hybrids. The first-wave release, an OPV, was limited in its potential impact.
- There is a large environmental effect on iron and zinc density, but the environmental factors are not yet well understood. Mineral density is not solely related to levels of micronutrients in the soil.
- The correlation between iron and grain yield is often negative, though only low to moderate and not always statistically significant. There is need for broader partnership and multi-environment data generation.

Table 2. First- and Second-Wave Cultivars Commercialized in India

Cultivar Name	Year	Iron Content* (% target)	Comments on Agronomic Properties
First Wave			
ICTP 8203-Fe	2012€	+24 ppm (92%)	2.2 t/ha grain yield (11% more than ICTP 8203); no change in zinc content; flowering time (45 days)
Second Wave			
Hybrid #7	2014¥	+19 ppm (86%)	3.6 t/ha grain yield (38% more than ICTP 8203); 36 ppm zinc content; flowering time 48 days (3 days later than ICTP 8203)
Hybrid #12	2014¥	+25 ppm (94%)	3.7 t/ha grain yield (41% more than ICTP 8203); 39 ppm zinc content; flowering time 48 days (3 days later than ICTP 8203)

*Average across two years (i.e. two rainy seasons), ICP-OES data. € Conducted in 42 locations during 2010 and 2011. ¥ Conducted in 31 locations during 2011 and 2012.

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2. Rai, KN; Govindaraj, M; Rao, AS. 2012. Genetic enhancement of grain iron and zinc content in pearl millet. *Quality Assurance and Safety of Crops & Foods* 4(3):119–125.
3. Paltridge, NG; et al. 2012. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. *Plant Soil* 361(1–2):251–260.

Vitamin A Maize

Thanda Dhliwayo, Natalia Palacios, Raman Babu, Felix San Vicente, & Kevin Pixley (CIMMYT)
Abebe Menkir, Bussie Maziya-Dixon, Oladeji Alamu, & Torbert Rocheford (IITA)

Table 1. Summary of Vitamin A Maize

Target Micronutrient		Vitamin A	
Target Country		Zambia. Secondary countries: Nigeria, Ghana	
Baseline Content		0 ppm	
Target Increment		+15 ppm	
Target Level in Crop		15 ppm	
Nutrition Factors		**Original Assumption**	**Measured/Revised[1]**
Maize Consumption, *grams/day (dry weight)*	Women	300 g/d	287 g/d
	Children	200 g/d	172 g/d
β-carotene Retention (%)		50%	37.5%
β-carotene Absorption (%)		8%	17%
Absorbed Vitamin A as % of EAR		50%	50%
Releases			
1st Wave	+6–8 ppm (40–60% target increment)	Released: Zambia, 2012; Nigeria, 2012; Ghana, 2012	
2nd Wave	+8–12 ppm (60–80% target increment)	Planned: Zambia, 2015; Ghana, 2015; Nigeria, 2015	
3rd Wave	>+15 ppm (>100% target increment)	Planned: 2017–18	

[1] Zambia

Breeding to Date

During HarvestPlus Phase I (Discovery, 2003–2008), initial screening of more than 1,500 maize germplasm accessions found ranges of 0–19 ppm provitamin A in existing maize varieties. Natural genetic variation in some lines exceeded the trial average by at least 60 percent for beta-carotene and provitamin A (1,2). These nutrients were consistently expressed in the maize inbred lines across different growing conditions, and further assessment indicated potential to increase the levels of multiple carotenoids simultaneously (1,2). Carotenoid degradation rate was also investigated. While not significant during grain/ear drying, carotenoid degradation occurs after three months during storage, and is higher in milled grain than in whole kernels (2, unpublished data). The rate of degradation is dependent on the genotype and can range from 60–90 percent after 12 months of storage (2, unpublished data).

In Phase II (Development, 2009–2013), the use of DNA-based techniques, such as association mapping studies, led to the identification of loci associated with provitamin A carotenoids and the development of DNA markers that have led to accelerated genetic gain in breeding for increased provitamin A content (3,4,5). The most important locus identified to date is the beta-carotene hydroxylase 1 (crtRB1). Validation experiments showed this rare allele often doubles, and sometimes triples, the total concentration of provitamin A carotenoid content in maize grain, mainly by increasing the content of beta-carotene (3). Provitamin A maize breeding programs at the International Maize and Wheat Improvement Center (CIMMYT), the International Institute of Tropical Agriculture (IITA), and the Zambia Agriculture Research Institute (ZARI) began in 2007 and have operated at full scale since 2011. The breeding pipeline includes materials from the two lead institutions, CIMMYT (tropical mid-altitude) and IITA (tropical lowlands), as well as local germplasm. Mainstreaming of provitamin A into product development is estimated at 10 percent for CIMMYT for the global maize breeding effort and 80 percent for the relevant mid-altitude target zone, and 40 percent for IITA.

Five hybrids and three synthetics were released in 2012, three in Zambia, four in Nigeria, and one in Ghana, all with 6–8 ppm provitamin A (about 50 percent of the target increment). The varieties combine competitive grain yield and strong farmer preferences in addition to higher provitamin A content in comparison to commercially available hybrids.

Future Releases

Eight hybrid leads and three open-pollinated varieties (OPVs) with up to 11 ppm provitamin A were submitted to the 2013/14 National Performance Trials (NPTs) in Zambia as well as in Ghana and Nigeria, and release of these second-wave varieties is expected in 2015. Third-wave hybrid leads are being evaluated at seven to nine sites in Zambia and Zimbabwe during 2013/14, and in Ghana and Nigeria in 2014/15. Most of these hybrids include lines carrying CrtRB1 and other alleles, with levels of provitamin A exceeding 15 ppm. Future breeding efforts focus on developing higher yielding, more robust hybrids exploiting specific adaptation for the different agroecological zones.

Capacity Building

Zambian capacity to conduct carotenoid analysis using high-performance liquid chromatography (HPLC) has been strengthened at ZARI, Mt. Makulu. Additional HPLC capacity was established by the HarvestPlus nutrition team at the Tropical Disease Research Centre (TRDC). To accelerate breeding at National Agricultural Research Systems (NARS) and seed companies, HarvestPlus/CIMMYT provides technical assistance and supports outsourcing of provitamin A molecular marker application and the use of double haploid production.

Regional Testing

HarvestPlus with CIMMYT and NARS partners expanded its regional testing and established an Elite Hybrid Trial in 2012 comprising released hybrids and leads along with respective inbred lines. NARS in Malawi, Zimbabwe, Ethiopia, Uganda, Democratic Republic of Congo, and Rwanda test different types of nurseries based on their demand. HarvestPlus with IITA has also organized regional variety and hybrid trials and dispatched them to partners in Benin, Ghana, Liberia, Sierra Leone, Mali, and Nigeria. Agronomic and provitamin A data from multiple sites per country allows high precision identification of fast-track candidates and inbred lines for breeding, as well as higher effectiveness in targeted breeding for yield and provitamin A stability based on adaptive pattern. By substituting temporal-by-spatial environmental variation in large-scale regional testing, testing steps can be eliminated and time-to-market shortened by 1-2 years. Vitamin A maize varieties are also being tested in Brazil, China, Colombia, India, Mozambique, and Panama.

Highlights

- Five hybrids and three OPVs have been released; these are competitive with commonly cultivated maize cultivars in grain yield and resistant to the prevalent major tropical diseases.
- Hybrids and OPVs with up to 15 ppm provitamin A content are in the development pipeline.
- CrtB1 markers are now being fully utilized in breeding at CIMMYT.
- In addition to breeding for provitamin A, both CIMMYT and IITA are also breeding for zinc content. Elite tropical maize inbred lines and hybrids with zinc content higher than 30 ppm have been identified in our breeding programs. Hybrids and OPVs have been developed from the best inbred lines and are being evaluated in multi-location trials in Central America and West Africa. Zinc maize varieties are being tested in Angola, Colombia, Guatemala, Honduras, Mexico, and Nicaragua.

Challenges

- The chemical mechanism of carotenoid degradation is not well understood. It may be possible to breed for decreased degradation rates in maize, or usefully exploit allelic variation for additional genes in the carotenoid pathway.

Table 2. Released Varieties of Vitamin A Maize

Release Name	Overall Average Yield[1]	Grain Texture	Provitamin A Content
Zambia – Released in 2012			
GV662A	3.86 t/ha	Semi flint	+7 ppm
GV664A	4.46 t/ha	Semi dent	+7 ppm
GV665A	3.85 t/ha	Flint	+8 ppm
Nigeria – Released in 2012			
Ife maizehyb-3	5.74 t/ha	Semi flint	+8 ppm
Ife maizehyb-4	5.20 t/ha	Semi flint	+8 ppm
Sammaz 38 (OPV)	3.54 t/ha	Semi flint	+6 ppm
Sammaz 39 (OPV)	3.56 t/ha	Semi flint	+7 ppm
Ghana – Released in 2012			
CSIR-CRI Honampa (OPV)	5.2t/ha		+6 ppm

[1] NPT data

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4. Yan, J; et al. 2010. Rare genetic variation at Zea mays crtRB1 increases b-carotene in maize grain. *Nat Genet* 42:322–329.
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Vitamin A Cassava

Peter Kulakow & Elizabeth Parkes (IITA)

Table 1. Summary of Vitamin A Cassava

<table>
<tr><td colspan="3">Target Micronutrient</td><td colspan="3">Vitamin A</td></tr>
<tr><td colspan="3">Target Countries</td><td colspan="3">Nigeria, Democratic Republic of Congo</td></tr>
<tr><td colspan="3">Baseline <i>(parts per million, ppm)</i></td><td colspan="3">0 ppm</td></tr>
<tr><td colspan="3">Target Increment</td><td colspan="3">+15 ppm</td></tr>
<tr><td colspan="3">Target Level in Crop</td><td colspan="3">15 ppm</td></tr>
<tr><td colspan="3">Nutrition Factors</td><td colspan="2">Original Assumption</td><td>Measured/Revised[1]</td></tr>
<tr><td colspan="2" rowspan="2">Cassava Consumption, <i>grams/day (fresh weight)</i></td><td>Women</td><td colspan="2">400 g/d</td><td>900 g/d</td></tr>
<tr><td>Children</td><td colspan="2">200 g/d</td><td>350 g/d</td></tr>
<tr><td colspan="3">β-carotene Retention (%)</td><td colspan="2">50%</td><td>21%</td></tr>
<tr><td colspan="3">β -carotene Absorption (%)</td><td colspan="2">8%</td><td>17%</td></tr>
<tr><td colspan="3">Absorbed Vitamin A as % of EAR</td><td colspan="2">50%</td><td>50%</td></tr>
<tr><td colspan="6">Releases</td></tr>
<tr><td>1st Wave</td><td colspan="3">+6–8 ppm (40–60% target increment)</td><td colspan="2">Released: Nigeria, 2011; DRC, 2008</td></tr>
<tr><td>2nd Wave</td><td colspan="3">+8–10 ppm (60–80% target increment)</td><td colspan="2">Planned: Nigeria, 2014; DRC, 2015</td></tr>
<tr><td>3rd Wave</td><td colspan="3">>+15 ppm (>100% target increment)</td><td colspan="2">Planned: 2016–18</td></tr>
</table>

[1] Nigeria

Breeding to Date

During HarvestPlus Phase I (Discovery, 2003–2008), initial screening of germplasm accessions found ranges of 0–19 ppm provitamin A in existing cassava varieties. Studies on genotype-by-environment (GxE) interaction for carotenoid content did not result in drastic changes in the relative ranking of genotypes, and heritability of carotenoid content in cassava roots is relatively high (1). The degradation rate of carotenoids was also investigated; sun drying was more detrimental to the provitamin A levels (44-73 percent degradation) in cassava than shade (41 percent) or oven drying (10-45 percent). *Gari*, the most popular cassava dish consumed in West Africa, had the highest provitamin A degradation of the foods tested (60-90 percent). The degradation of staple crops during storage reached levels as high as 80 percent after 1-4 months of storage and was highly dependent on genotype (2).

In Phase II (Development, 2009–2013), HarvestPlus and its partners developed analytical methods for cassava screening, demonstrating that spectrophotometric screening overestimated high-performance liquid chromatography (HPLC) values in yellow-fleshed cassava (3). Rapid-cycling recurrent selection was used to shorten the normal breeding cycle from eight to two to three years for high carotenoid content (4). Breeding programs for provitamin A cassava at the International Center for Tropical Agriculture (CIAT) and the International Institute of Tropical Agriculture (IITA) assumed full operational scale by 2011. CIAT generates high-provitamin A sources via rapid cycling in pre-breeding and provides in vitro clones and seed populations to IITA and the Nigerian National Root Crops Research Institute (NRCRI) and the Institut National pour l'Etude et la Recherche Agronomiques (INERA) in the Democratic Republic of Congo (DRC) for local adaptive breeding. Mainstreaming of the provitamin A trait is estimated at 50 percent for the IITA cassava breeding effort, and 30 percent for CIAT.

Three first-wave vitamin A cassava varieties with 6–8 ppm provitamin A were released in 2011. Five second-wave varieties with up to 10 ppm are in the final stages of evaluation before official release in Nigeria. In DRC, a variety developed by IITA under HarvestPlus and officially released as I011661 in 2008 was shown to contain 7 ppm provitamin A and is now under multiplication/distribution.

Future Releases

More than 50 provitamin A varieties are now at different stages of evaluation to identify those that are agronomically competitive for third-wave release. The top five leads have up to 15 ppm (greater than 100 percent of target increment). These varieties will be put in tissue culture for international distribution, particularly targeting potential expansion countries. Some National

Agricultural Research Systems (NARS) have started their own programs to release new varieties from past introductions; the most recent release developed by IITA is I06/1635 in Sierra Leone.

Capacity Building

Near-infrared reflectance spectroscopy (NIRS) was provided to IITA to accelerate and increase the quality and reliability of measuring and comparing Total Carotenoid Content (TCC) in breeding germplasm. In addition to NIRS, a portable device known as iCheck™ Carotene, used for measuring carotenoid levels, was introduced and has provided useful rapid field evaluation and selection of genotypes in early breeding stages. The correlation between iCheck™ and spectrophotometer is high enough to produce acceptable data.

Regional Testing

IITA distributes elite provitamin A clones to numerous countries in the region. Local GxE testing of the deployed clones provides information on provitamin A levels and agronomic performance from multiple sites per country and allows high-precision identification of fast-track candidates and parents for breeding, as well as greater effectiveness in targeted breeding based on adaptive pattern. Vitamin A cassava varieties are also being tested in Brazil, Central African Republic, Colombia, Ethiopia, Ghana, Cote D'Ivoire, Kenya, Malawi, Mozambique, Sierra Leone, Tanzania, and Uganda.

Highlights

- Three first-wave varieties were released in Nigeria in 2011; second-wave varieties will be released in 2014.
- One first-wave variety was identified in DRC for multiplication and distribution; promising second-wave varieties are in testing.
- Rapid-cycling recurrent selection has been implemented, and clones with up to 15 ppm provitamin A content are in the development pipeline.

Challenges

- Dry matter content for provitamin A varieties is somewhat low compared to local preference and is a priority for improvement.
- Root mealiness or poundability, important in many African diets, is limited in current varieties and will be beneficial for provitamin A retention in cooking and development of weaning foods for children. This is also a breeding priority.

Table 2. Released Varieties of Vitamin A Cassava

Variety Name	Total Carotenoid Content (FW)*	Fresh Root Yield	Yield Relative to Check	Dry Matter
Nigeria – Released in 2011				
TMS 01/1371	+8 ppm	20.1 t/ha	87%	30.7%
TMS 01/1412	+7 ppm	29.8 t/ha	128%	30.1%
TMS 01/1368	+7 ppm	26.7 t/ha	115%	33.4%
30572 (Control)	*+0.9 ppm*	*23.2 t/ha*	*100%*	*37.1%*
DRC – Released in 2008				
I011661	+9 ppm	34.9 t/ha	NA	30%
Butamu (Check)	*+4.4 ppm*	*35.0 t/ha*	*NA*	*35%*

* Provitamin A content is approximately 80% of total carotenoid content (fresh weight – FW)

Notes: Data from two years of multi-locational NCRP testing at 9 sites during 2008/09 and 2009/10 (Nigeria).

1. Ssemakula, G; Dixon, AGO; Maziya-Dixon, B. 2007. Stability of total carotenoid concentration and fresh yield of selected yellow-fleshed cassava (Manihot esculenta Crantz). *Journal of Tropical Agriculture* 45(1-2):14–20.
2. De Moura, F; Miloff, A; Boy, E. 2013. Retention of provitamin A carotenoids in staple crops targeted for biofortification in Africa: Cassava, maize and sweet potato. *Critical Reviews in Food Science and Nutrition*.
3. Kimura, M; et al. 2007. Screening and HPLC methods for carotenoids in sweetpotato, cassava and maize for plant breeding trials. *Food Chemistry* 100(4):1734–1746.
4. Ceballos, H; et al. 2013. Rapid cycling recurrent selection for increased carotenoids content in cassava roots. *Crop Science* 53(6):2342–2351.

Vitamin A Orange Sweet Potato (OSP)

Maria Andrade (CIP)

Table 1. Summary of Vitamin A OSP

Target Micronutrient		Vitamin A	
Target Countries		Uganda (HarvestPlus), Southern Africa (CIP)	
Baseline *(parts per million, ppm)*		2 ppm	
Target Increment		+30 ppm	
Target Level in Crop		32 ppm	
Nutrition Factors		**Original Assumption**	**Measured/ Revised[1]**
Sweet potato Consumption, *grams/day (fresh weight)*	Women	200 g/d	200 g/d
	Children	100 g/d	75 g/d
β-carotene Retention (%)		50%	75% (boiled)
β-carotene Absorption (%)		8%	7%
Absorbed Vitamin A as % of EAR		50%	>90%
Releases			
Full Target Varieties	+39-95	Uganda: 2004, 2007 (*See chart below for additional releases)*	

[1] Uganda

Breeding to Date

Sweet potato is widely consumed in Africa south of the Sahara (1). Conventionally bred orange sweet potato (OSP) containing vitamin A was the first biofortified crop released by HarvestPlus and its partners. Plant breeders have produced several OSP varieties with provitamin A content of 30–100 ppm, exceeding the target level of 32 ppm. Analytical methods for sweet potato were developed; in orange and salmon-fleshed sweet potatoes, high-performance liquid chromatography (HPLC) and spectrophotometric screening resulted in similar quantifications of beta-carotene (2). Breeding research in Uganda is conducted by the National Crops Resources Research Institute (NaCRRI) with the support of the International Potato Center (CIP). Breeding for provitamin A OSP at both NaCRRI and CIP has assumed full operational scale. The full breeding pipeline consists of both locally developed germplasm and introductions from CIP. NaCRRI is engaged in testing biofortified candidate varieties and providing other technical support to seed systems. As the provitamin A trait is mainstreamed in breeding populations, ongoing OSP breeding concentrates on tolerance to biotic and abiotic stress while maintaining/enhancing provitamin A levels. HarvestPlus coordinates with NaCRRI and CIP to ensure a continuous flow of improved varieties for Uganda. In addition to the two landraces (Ejumula and Kakamega), which were identified and released prior to the start of the HarvestPlus activities in Uganda, two OSP varieties with the full provitamin A target were released in 2007. In 2013, two clones (SPKOO4/2006/1136 and NAS7/2006/292) were pre-released.

Future Releases

New OSP varieties are subject to both on-station and multi-location treatment as part of the release process. These will be further taken for on-farm trials with farmers participating in the project so that palatability and acceptance tests can be conducted before release and bulking of vines. Biofortified varieties are now being introduced in many parts of Africa and South America, as well as China. In 2009, CIP launched its Sweet Potato for Profit and Health Initiative (SPHI), which seeks to deliver OSP to 10 million households in Africa by 2020. Eight countries in Africa have released 46 improved sweet potato varieties since 2009, of which 31 are OSP. Helen Keller International (HKI) has integrated biofortification into programs to combat vitamin A deficiency, promoting OSP through nutrition education and homestead food production. Varieties have been released in Angola, Brazil, Burkina Faso, China, Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mozambique, Nicaragua, Niger, Nigeria, Rwanda, Senegal, South Africa, Syria, Uganda, and Zambia.

Highlights & Challenges

- OSP is a widely supported intervention throughout Africa south of the Sahara, and farmers are adopting OSP varieties.
- Extensive evidence on the impact of consuming OSP on vitamin A intake and status of women and children has been produced.
- Dry matter content for OSP varieties is somewhat low compared to local preference.

Table 2. Released Varieties of Vitamin A OSP

Variety Name	Mean Yield(tons/hectare)	Dry Matter (%)	Beta-carotene (ppm)
Ghana			
Cri-Bohye	22	31	3760–7230
Madagascar			
199062.1 Cri-Bohye In Ghana	22	31	3760–7230
Ejumula	14.7	33.0	7760–14370
Resisto	15.8	24	24900
Zambezi	15.1	28.5	10900
Malawi			
Ana Akwanire	25	29.0	55
Kadyaubwerere	35	31.1	89
Kaphulira	35	30	32

Mathuthu	25	29	29
Zondeni	8–16	30–32	90
Mozambique			
199062.1 Cri-Bohye In Ghana	22	31	38–72
Amelia	17.3	32.1	50
Bela	25.9	27.5	84
Coromex	15.3	22.7	110
Cecilia	18.3	26.7	60
Cn-1424-9	20	27	110
Cn-1448-49	15.7	22.7	45–49
Delvia	23.4	32.8	55
Ejumula	14.7	33.0	78–144
Erica	16.7	25.6	102
Esther	18.6	29.6	49
Gaba Gaba	6.5	23.9	110
Ininda	22.2	29.3	53
Irene	19.6	28.8	83
Jane	21.2	29.2	56
Japon Tresmesino Selecto	14.5	21.6	38–72
Jewel	21	28	110
Kandee	14.5	25.3	110
Lourdes	18.3	25.8	99
Lo-323	13.6	21	55
Melinda	27.1	23.6	57
Namanga	19.3	27	84
Persistente MGCL01	5	37	110
Resisto	15.8	24	249
Sumaia	21.6	25.2	77
Tainung 64	15	23	38–72
Tio Joe	20	26.7	103
Nigeria			
Umuspo/1	63.6	39.3	70
Umuspo/3(Mother’s Delight)	31.4	28.7	30
Rwanda			
Ejumula	14.7	33.0	78–144
Kakamega Spk004	16.5	32	38
Rw11-2560	20	21	105
Rw11-2910	20	31.1	41
South Africa			
Impilo	31.1	21.4	29–70
Khano	24.5	18.2	120–156
Resisto	15.8	24	249
W-119	19.5	25	88–130
Ejumula	14.7	33.0	7760–14370
Kakamega Spk004	16.5	32	38
Kenspot-3	18.7	32.5	1380
Kenspot-4	17.1	30.4	3960
Kenspot-5	14.8	25.9	5490
K566632	15–20	25–26	700–800
W151	18	28	10500–14370
Tanzania			
Carrot C	15.0	33.0	123–143
Ejumula	14.7	33.0	78–144
Kakamega Spk004	16.5	32	38
Kiegea Kbh2001/261	13	25–30	73
Matayakbh2001/261	13	25–30	73
Mayai	10	32.5	110
Uganda			
Ejumula	14.7	33.0	78–144
Kakamega Spk004	16.5	32	37
Naspot 8	20	32.5	28
Naspot 10	16	30.5	110
Naspot 12	20	30	72
Naspot 13	18	28	110
Zambia			
Chiwoko	20.0	34	11030
Olympia	25.5	31	TBD
Twatasha	20	31	TBD
Zambezi	15.1	28.5	10900

1. Woolfe, JA. 1992. *Sweet potato: An untapped food source*. Cambridge, UK: Cambridge University Press
2. Kimura, M; et al. 2007. Screening & HPLC methods for carotenoids in sweetpotato, cassava & maize for plant breeding trials. *Food Chemistry* 100(4):1734–1746.

PROGRESS BRIEF #8

CROP DEVELOPMENT

Vitamin A Banana/Plantain

Beatrice Ekesa (Bioversity International – Uganda)

Table 1. Summary of Vitamin A Banana/Plantain

Target Micronutrient	Vitamin A		
Target Countries	Democratic Republic of Congo (DRC), Burundi		
Baseline *(parts per million, ppm; fresh weight – FW)*	10–18 ppm (1)		
Target Increment	+58 ppm		
Target Level in Crop	17–106 ppm (1)		
Nutrition Factors		**Original Assumption**	**Measured/ Revised**
Banana/Plantain Consumption, *grams/day (FW)*	Women	500 g/d	700–1,100 g/d (2)
	Children	200 g/d	250 g/d (unpublished)
β-carotene Bioaccessibility (%)*	ABB Plantain	8%	16% (3)
	East African Highland Bananas (EAHBs)	8%	27% (3)
Releases			
Fast-track Identified	17–106 ppm	DRC, Burundi – official release of 5 varieties planned in 2014	
2nd Wave	At least 4 varieties	Planned 2016	

***Bioaccessibility refers to the amount of the β-carotene available for absorption after digestion; bioavailability data, which measures the amount digested, absorbed, and utilized, is not yet available.

Breeding to Date

Breeding banana/plantain (*Musa*) is complex, as commercial varieties are sterile triploids (3X). Among the fertile groups, a high degree of cross incompatibility can exist. Further, the *Musa* crop cycle is long. Initially, high-provitamin A African *Musa* varieties adapted under relevant conditions in African target countries were evaluated and deployed to farmers along with crop management recommendations. In the longer term, breeding combines the best provitamin A sources with African elite varieties, which carry the productivity, disease and virus resistance, and sensory traits that farmers prefer.

During HarvestPlus Phase I (Discovery, 2003–2008), initial screening of more than 300 genotypes found 1–345 ppm provitamin A in existing banana/plantain varieties. Carotenoid content was indicative of pulp color, and maximum values for provitamin A carotenoids (pVACs) were discovered in African varieties. In general, about half of the provitamin A content is in the form of alpha-carotene, which is estimated to have a retinol equivalence of 24:1 (beta-carotene is 12:1). Adapted genotypes were evaluated for use of parents in multi-location trials in Nigeria and Cameroon, and results indicated stability for provitamin A carotenoids across environments.

Since 2006, Bioversity International has continued work on vitamin A banana/plantain. Completed activities include: germplasm screening of over 400 accessions from different regions; identification of proteins and enzymes responsible for the accumulation of pVACs in fruit of nutritionally rich *Musa* cultivars; a genome-wide study of the main gene families involved in biogenesis carotenoid pathways; and, studies for nutritional profiling and bioaccessibility of pVACs from *Musa*-based dishes.

Comparison of conventional (left) and vitamin A banana (right). Photo by B. Ekesa

Within eastern Africa, trials were established of *Musa* cultivars of different sub-groups (plantain, East African Highland bananas, ABB cooking bananas, AA and AAA dessert bananas, Pacific plantains, and AA cooking bananas) with total beta-carotene (t-BC) equivalents of 40–95 ppm, giving a retinol activity equivalent (RAE)[1] of more than 333 micrograms (µg) per 100 grams

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[1] A measure of vitamin A activity based on the capacity of the body to convert provitamin carotenoids containing at least one unsubstituted ionone ring to retinaldehyde. (1 microgram RAE = 1 mg retinol = 12 mg β-carotene = 24 mg other vitamin A precursor carotenoids).

(g) of dry weight. Evaluation of pVAC content has gone hand-in-hand with evaluation of agronomic performance. In addition, sensory/organoleptic evaluation is also ongoing.

Preliminary findings indicate that at least five genotypes have potential to perform well within eastern Africa. Available results from sensory/organoleptic trials show that the introduced cultivars fare well in hedonic tests, and overall acceptance of the introduced cultivars did not significantly differ from that of local cultivars. The mean total pVACs ranged from 17–106 ppm, and a significantly higher level of pVACs was observed as the fruit developed from unripe (ripening stage 1) to ripe (ripening stage 5). Six out of nine cultivars can meet more than 100 percent of the vitamin A estimated average requirement (EAR) for children (1–5 years), and four out of nine cultivars meet more than 90 percent of the EAR for women when 100 g of fruit at ripening stage 5 are consumed. If adopted, consumption of the fruit itself or products derived from the cultivars could contribute substantially to the vitamin A intake of vulnerable population groups, such as children aged 6–59 months and women of reproductive age.

Future Releases

Of the 12 first-wave varieties, five are being multiplied and will be officially released in DRC and Burundi between June and October 2014.

Four second-wave varieties high in pVACs have been recently identified. They will be ordered from the International Musa Germplasm Transit Centre (ITC) collection, multiplied, and tested for their agronomic performance and acceptability within eastern Africa.

Regional Testing

The five cultivars preferred in DRC and Burundi will also be tested through other research projects in Uganda and Tanzania. Vitamin A bananas are also being tested in Cameroon, Cote d'Ivoire, and Nigeria.

Highlights

- Fast-track varieties with high levels of provitamin A have been identified and are being tested (agronomic, organoleptic) by farmers in DRC and Burundi. A significant proportion of these varieties are likely to be incorporated within existing farming systems.
- Four second-wave varieties have been identified, and they will be ordered, multiplied, and trials established to test their agronomic performance and acceptability in eastern Africa.

Challenges

- The yield (bunch size) of vitamin A-rich varieties is relatively low compared to local varieties within similar genomic groups.
- The process of ordering, tissue multiplication, trial establishment, and continued evaluation is often longer than planned.

Table 2. Varieties Selected for Dissemination in Eastern DRC and Burundi

Variety Name	Country of Origin	Genome-Sub group	Fruit Ripening Stage	Total Carotenoid Content (FW)*
Apantu	Ghana	AAB-Plantain	Unripe	46.83 ppm
			Ripe	100.71 ppm
Bira	Papua New Guinea	AAB-Pacific plantain	Unripe	43.42 ppm
			Ripe	106.38 ppm
Pelipita	Philippines	ABB-Plantain	Unripe	25.35 ppm
			Ripe	17.44 ppm
Lai	Thailand	AAA-Dessert	Unripe	nd
			Ripe	nd
To'o	Papua New Guinea	AA-Dessert	Unripe	5.60 ppm
			Ripe	77.69 ppm

*Measures of fruit samples obtained from North Kivu, fresh weight (FW) determined by establishing moisture content following measurement of fresh sample and freeze-dried sample [value of dry matter/ (100/100-moisture %)]. nd= No data because To'o mature fruit was not available in North Kivu during sample collection thus not analyzed at the moment; A=Acuminata, AA= Diploid Acuminata, AAA=Triploid Acuminata, B=Balbisiana, BB=Diploid Balbisiana

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PROGRESS BRIEF #9

CROP DEVELOPMENT

Iron and Zinc Lentils

Ashutosh Sarker (ICARDA)

Table 1. Summary of Iron and Zinc Lentils

Target Micronutrient		Iron. Secondary: zinc	
Target Countries		Bangladesh, Nepal, India	
Baseline *(parts per million, ppm)*		40 ppm	
Target Increment		+30 ppm	
Target Level in Crop		70 ppm	
Nutrition Factors		**Original Assumption**	**Measured/Revised**
Lentil Consumption, *grams/day (dry weight)*	Women	50 g/d	40 g/d
	Children	25 g/d	20 g/d
Iron Retention (%)		85%	90%
Iron Absorption (%)		Not known	Not known
Absorbed Iron as % of EAR		Not known	Not known
Releases			
India		L4704	125 ppm Fe, 74 ppm Zn
Nepal		ILL 7723	83 ppm Fe, 61.5 ppm Zn
Bangladesh		Barimasur-7	81 ppm Fe

Breeding to Date

The International Center for Agricultural Research in the Dry Areas (ICARDA) leads research to biofortify lentils with higher levels of iron (Fe) and zinc (Zn). A large number of breeding lines (more than 1,600), landraces, and released varieties have been analyzed for iron and zinc content. Iron content ranged from 42–132 ppm and zinc content ranged from 23–78 ppm. Iron and zinc were analyzed at Waite Institute, Australia; University of Saskatchewan, Canada; Indian Agricultural Research Institute, India; and North Dakota State University, USA.

Several released varieties that possess high iron and zinc levels and have good agronomic performance have been identified. These varieties are in fast-tracking and include:

- Bangladesh: Barimasur-4 (86.2 ppm Fe), Barimasur-5 (86 ppm Fe, 59 ppm Zn), Barimasur-6 (86 ppm Fe, 63 ppm Zn), and Barimasur-7 (81 ppm Fe);
- Nepal: Sisir (98 ppm Fe, 64 ppm Zn), Khajurah-2 (100.7 ppm Fe, 59 ppm Zn), Khajurah-1 (58 ppm Zn), Sital (59 ppm Zn), Shekhar (83.4 ppm Fe), and Simal (81.6 ppm Fe);
- India: Pusa Vaibhav (102 ppm Fe);
- Syria/Lebanon: Idlib-2 (73 ppm Fe) and Idlib-3 (72 ppm Fe); and
- Ethiopia: Alemaya (82 ppm Fe, 66 ppm Zn)

Since 2009, seed multiplication, large-scale demonstrations, and seed dissemination have been prioritized. Farmers, including women farmers, have participated in capacity development and awareness programs.

In parallel to the identification of fast-track varieties, parents with high iron and zinc were identified and have been used in cross-breeding programs at ICARDA, Bangladesh Agricultural Research Institute (BARI), Nepal Agricultural Research Council (NARC), and Indian Agricultural Research Institute (IARI). Final, intermediate, and primary products have been developed and are under evaluation for yield traits and micronutrient levels. Identification of genetically fixed lines and germplasm with high levels of iron and zinc at ICARDA helped to develop new international nurseries for red lentils and green lentils (Lentil International Elite Nursery-Micronutrient). These nurseries (LIEN-MN-R and LIEN-MN-Y) have been shared with 14 national programs. Additionally, recombinant inbred lines with a sufficient number of progenies are in F7/F6 stages for genetic studies.

Multi-location testing is strong, and varieties/advanced lines have been tested in Bangladesh, Ethiopia, India, Nepal, and Syria. Significant genotype-by-environment (GxE) interaction was observed in many cases; iron content is more sensitive to environmental fluctuations compared to seed zinc content. A few genotypes were identified with stable high-iron and zinc contents (IPL 320, L4704).

In India, one high-iron and zinc line, L4704 (125 ppm Fe and 74 ppm Zn) has been registered by the National Bureau of Plant Genetic Resources. In Bangladesh, Barimasur-7 was released for high iron (81 ppm).

Future Releases

In Nepal, ILL 7723 has been recommended by the National Variety Release Committee and is expected to be released in 2014. Additionally, a proposal for RL-12 is under preparation and will be submitted by mid-2014.

Regional Testing

High-iron and zinc content lines with excellent agronomic performance are selected from the ICARDA international nursery and national breeding programs of Bangladesh, India, and Nepal and are subject to regional testing. Iron lentils are also being tested in Ethiopia and Syria.

Highlights

- Screening of a large number of germplasm, breeding lines, and varieties led to the identification of several released varieties high in iron and zinc; these are in fast-tracking.
- Farmers and consumers are increasingly aware of the value of daily diets rich in iron and zinc and the health benefits of lentils. Enthusiasm for growing high-iron and zinc varieties and consuming them is increasing.
- Identification of high-iron and zinc genotypes has encouraged breeders to use these in hybridization programs.
- High-iron and zinc lentils are available to consumers in Bangladesh, Nepal, India, and Syria/Lebanon.

Challenges

- The production of sufficient quantities of quality seed of high-iron and zinc varieties.
- The development of high-yielding and high-micronutrient varieties with stable performance across environments.
- The understanding of correlation of iron and zinc levels with other macro- and micronutrients in lentil seeds.

Table 2. Released Varieties of Iron and Zinc Lentils

Variety Name	**Iron Content**	**Zinc Content**
India – Released in 2012		
L4704	+85 ppm	74 ppm
Nepal - Released in 2013		
ILL 7723	+43 ppm	61.5 ppm
Bangladesh – Released in 2013		
Barimasur-7	+41 ppm	NA

Iron and Zinc Irish Potato

Merideth Bonierbale (CIP)

Table 1. Summary of Iron and Zinc Irish Potato

Target Micronutrient		Iron (Zinc)	
Target Countries		Rwanda, Ethiopia	
Baseline *(parts per million, ppm; dry weight, DW)*		19 ppm (14 ppm)	
Target Increment		+29 ppm (+19 ppm)	
Target Level in Crop		48 ppm (33 ppm)	
Nutrition Factors		**Original Assumption**	**Measured/Revised[1]**
Potato Consumption, *grams/day (fresh weight)*	Women	400 g/d	
	Children	200 g/d	
Iron (Zinc) Retention (%)		50%	80%
Iron (Zinc) Absorption (%)		10% (25%)	
Absorbed Iron (Zinc) as % of EAR		50% (40%)	
Releases			
1st Wave	+25 ppm iron, +19 ppm zinc	Planned: Rwanda and Ethiopia, 2017	

[1] CIP

Breeding to Date

Initial screening of germplasm accessions found ranges of 11-30 ppm iron and 8-25 ppm zinc in existing potato varieties. Levels of vitamin C and phenolic compounds were also assessed, as these affect iron absorption. Studies on genotype-by-environment (GxE) interaction for iron and zinc found significant effects, but these did not result in drastic changes in the relative ranking of genotypes (1). Heritability of iron and zinc concentrations in potato tubers is moderately high (2), and no negative correlation was found between micronutrient concentration and important resistance traits. There is some evidence that iron and zinc concentration may have an effect on tuber yield; further research is needed.

Mineral retention during cooking has been examined; cooking resulted in no significant differences in mineral levels between raw and cooked potatoes, and mineral determinations on raw potatoes may be used directly (3). To maximize the nutrition benefits of iron and zinc dense potato, promoters and inhibitors of absorption, either in the staple crop or in the accompanying diet, should also be considered. Vitamin C, or ascorbic acid, is a promoter of iron absorption present in potatoes. Cooking degrades ascorbic acid and therefore affects potential mineral absorption. The magnitude of this effect varies by genotype and cooking method with some varieties retaining over 70 percent of their vitamin C when boiled (4).

Since 2009, a number of lines have been identified that express more than 60 percent of the iron target and 75 percent of the zinc target levels. Inductively coupled plasma (ICP) was identified as the gold standard for high-precision mineral analysis capable of detecting contamination with soil or in the lab. X-ray fluorescence (XRF) spectrometry calibrations and standards have been developed for high-throughput screening. Breeding programs for iron and zinc potato at the International Potato Center (CIP) have assumed full operational scale. CIP generates high iron and zinc sources and provides clones and seed populations to the Rwanda Agricultural Board (RAB) and the Ethiopian Institute for Agriculture Research (EIAR) for local adaptive breeding.

Samples of the base population with high levels of iron and zinc and improved cycles have been introduced in Ethiopia and Rwanda for participatory selection and further enhancement by breeding. In 2014, the best-performing and farmer-preferred clones will be selected for fast-track delivery, with official release expected by 2017.

In Nepal, ILL 7723 has been recommended by the National Variety Release Committee and is expected to be released in 2014. Additionally, a proposal for RL-12 is under preparation and will be submitted by mid-2014.

Future Releases

More than 20 iron and zinc potato clones are now at different stages of evaluation to identify those that are agronomically competitive for release. The top leads have up to 35 ppm iron and 29 ppm zinc. These varieties will be put in tissue culture for international distribution to additional potential target countries, Malawi, Nepal, Bhutan and Bangladesh. Assessment of levels achieved under local conditions is pending.

Capacity Building

In 2011, RAB's analytical capacities were strengthened by installing and implementing X-ray fluorescence (XRF) machines for in-country mineral analysis. Though installed primarily for analysis of iron beans, the machines can also be used for potato analysis. Staff from Rwanda and Ethiopia have been trained on potato sampling and analysis techniques.

Regional Testing

CIP distributes elite iron and zinc clones to countries in the region. Local GxE testing of the deployed clones provides information on mineral levels and agronomic performance from multiple sites per country. It also allows high-precision identification of fast-track candidates and parents for breeding as well as greater effectiveness in targeted breeding based on adaptive pattern.

Highlights

- Clones with 60 percent of the iron target and 75 percent of the zinc target are in the development pipeline.
- On-farm evaluations began in Ethiopia in 2012 and in Rwanda in 2013.

Challenges

- Standardization of mineral analysis in target countries is a challenge; additional capacity is needed to prevent soil contamination of samples.
- Human absorption of potato iron and zinc needs to be determined to inform biofortification for impact.
- Further information is needed on the content of absorption enhancers and inhibitors in promising varieties.

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Iron Cowpea

B.B. Singh (G.B. Pant University of Agriculture and Technology)

Table 1. Summary of Iron Cowpea

Target Micronutrient		Iron	
Target Countries		India	
Baseline *(parts per million, ppm; dry weight, DW)*		30 ppm	
Target Increment		+33 ppm	
Target Level in Crop		63 ppm	
Nutrition Factors		**Original Assumption**	**Measured/Revised***
Cowpea Consumption, *grams/day (fresh weight)*	Women	200 g/d	
	Children	100 g/d	
Iron Retention (%)		85%	
Iron Absorption (%)		5%	
Absorbed Iron as % of EAR		30%	
Releases			
Pant Lobia-1		India, 2008	82 ppm Fe, 40 ppm Zn
Pant Lobia-2		India, 2010	100 ppm Fe, 37 ppm Zn
Pant Lobia-3		India, 2013	67 ppm Fe, 38 ppm Zn
Pant Lobia-4		India, 2014	51 ppm Fe, 36 ppm Zn

*Original assumptions have not yet been measured and revised.

Breeding to Date

During HarvestPlus Phase I (Discovery, 2003-2008), the International Institute of Tropical Agriculture (IITA) screened and assessed more than 2,000 cowpea lines in replicated screening in Nigeria. Screening activities identified minerals and agronomic traits, and assayed subsets of materials for protein and total carotenoids, finding iron content ranging from 27-97 ppm and zinc content from 23-62 ppm. Genetic variation for minerals suggests that target increments are feasible. A cowpea sampling protocol to reduce iron contamination from dust was developed and implemented.

Germplasm lines (1,541) of different origins were obtained from the genetic resources unit at IITA and sown in the experimental field in Minjibir, Kano State, Nigeria. The grain was analyzed for protein and nine mineral contents, then researchers used cluster analysis to group the cowpea germplasm accessions based on their levels of protein and mineral concentrations to identify promising parent lines. A significant and positive relationship between protein and iron concentration in grain was demonstrated (1).

In HarvestPlus Phase II, cowpea research shifted to G.B. Pant University of Agriculture and Technology, Pantnagar, India. It focused on the introduction and further improvement of recently developed photo-insensitive and heat-tolerant “60-day cowpea” varieties by IITA. 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. They were subsequently notified in 2009 and 2011 by the national Central Sub-Committee on Standards, Notification and Release of Varieties. Pant Lobia-3 was released in 2013. These varieties have now entered the national seed multiplication system and seed is available to farmers.

The National Meeting on Arid Zone Legumes in June 2014 identified another variety, Pant Lobia-4, for release in 2014.

Future Releases

Several varieties with high iron and zinc are in advanced variety trials at state and national levels. More than 100 new breeding lines with 60-65 day maturity, combining high yield and resistance to cowpea yellow mosaic, have been developed and are being tested in multi-location trials in eastern and southern India. The best varieties in advanced trials have up to 66 ppm iron and 60 ppm zinc.

Highlights

- Three iron cowpea varieties have been released in India, and a fourth identified for release in 2014.

Challenges

- As Green Revolution-led ‘wheat-rice’ and ‘rice-rice’ cropping systems have become more popular, legume production has been pushed to marginal lands, resulting in stagnant production of pulses and high prices.
- Introducing short duration cowpea varieties as a niche crop in the ‘wheat-rice’ and ‘rice-rice ’cropping systems has potential, but it is not well known to farmers.
- In addition to the current work on wheat-rice-cowpea system in northern India and as a multiple crop throughout India, a collaborative project with the International Rice Research Institute (IRRI) has also been initiated to demonstrate these new varieties in rice-rice cropping systems in mid and south India.
- The new cowpea varieties are also being introduced as a niche crop for the late *Kharif* (rainy) season planting after the harvest of fodders and maize crop for green cobs. The potential area under “wheat-cowpea-rice” system in northern India is about 10 million hectares; a similar potential area exists for the “rice-cowpea-rice” system. Even a partial success in introducing short duration cowpea varieties in these systems would bring India close to meeting its pulses requirement in the near future.

Table 2. Released Varieties of Iron Cowpea

Variety Name	Release Year	Iron Content	Zinc Content	Av. Yield	Comments on Agronomic properties
Pant Lobia-1	2008	82 ppm Fe	40 ppm Zn	1500kg/ha	Early erect plants, multiple disease res., white seeds
Pant Lobia-2	2010	100 ppm Fe	37 ppm Zn	1500Kg/ha	Early erect plants, multiple disease res., red seeds
Pant Lobia-3	2013	67 ppm Fe	38 ppm Zn	1500Kg/ha	Early semi erect, multiple disease res., brown seeds
Pant Lobia-4	2014	51 ppm Fe	36 ppm Zn	1700kg/ha	Early semi erect, multiple disease res., brown seeds
Buksora local	-	26 ppm Fe	30 ppm Zn	800Kg/ha	Medium semi-erect , virus susc., red specs on seeds

Notes: Data from 3-4 years of multi-locational testing at 8-10 sites

Figure 1. Pant Lobia-2 (top with red seeds) and Pant Lobia-1 (bottom with white seeds)

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Iron and Zinc Sorghum

Ashok Kumar

Table 1. Summary of Iron and Zinc Sorghum

<table>
<tr><td colspan="3">Target Micronutrient</td><td colspan="4">Iron, Zinc</td></tr>
<tr><td colspan="3">Target Countries</td><td colspan="4">India, Mali</td></tr>
<tr><td colspan="3">Baseline <i>(parts per million, ppm; dry weight, DW)</i></td><td colspan="4">30 ppm iron, 20 ppm zinc</td></tr>
<tr><td colspan="3">Target Increment</td><td colspan="4">+30 ppm iron, +12 ppm zinc</td></tr>
<tr><td colspan="3">Target Level in Crop</td><td colspan="4">60 ppm iron, 32 ppm zinc</td></tr>
<tr><th colspan="2">Nutrition Factors</th><th>Original Assumption</th><th colspan="4">Measured/Revised[1]</th></tr>
<tr><td rowspan="2">Sorghum Consumption, <i>grams/day</i></td><td>Women</td><td>200 g/d</td><td colspan="4">300 g/d</td></tr>
<tr><td>Children</td><td></td><td colspan="4">150 g/d</td></tr>
<tr><td colspan="2">Iron Retention (%)</td><td>85%</td><td>Iron Retention (%)</td><td>85</td><td>Zinc Retention (%)</td><td>90</td></tr>
<tr><td colspan="2">Iron Absorption (%)</td><td>5%</td><td>Iron Absorption (%)</td><td>5</td><td>Zinc Absorption (%)</td><td>20</td></tr>
<tr><td colspan="2">Absorbed Iron as % of EAR</td><td>30%</td><td>Absorbed Iron as % of EAR</td><td>30</td><td>Absorbed Zinc as % of EAR</td><td>26</td></tr>
</table>

[1] India

Breeding to Date

During HarvestPlus Phase I (Discovery, 2003-2008), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) screened and assessed more than 2,200 sorghum lines, finding iron concentrations ranging from 20-70 ppm and zinc concentrations of 13-47 ppm. Levels of anti-nutritional factors, like tannin and phytate, were also analyzed for the prospect of breeding high iron and zinc cultivars with lower levels of such compounds (1). Agronomic fortification—basal and foliar application of micronutrient fertilizers—was tested but did not appreciably increase the grain iron and zinc concentrations.

Iron and zinc density is highly heritable, and is predominantly under additive genetic control. There is no penalty shown in agronomic traits when combined with high iron and zinc concentration (2). Following initial screening, promising hybrid parents and hybrids were identified. Several existing commercial hybrids were shown to have high iron and zinc concentrations and could be used for fast-track dissemination. Currently, released variety PVK 801 is being promoted among farmers in Maharashtra. Additionally, new hybrids have been developed and are in multi-locational trials in India (3).

In Mali, promising genotypes are being validated for iron and zinc concentrations. Progenies derived from high iron Guinea landrace donor parents and from the Diversified Dwarf Guinea Population are expected to provide novel diversity for micronutrient concentration, as well as achieve acceptable plant height.

Future Releases

One promising variety and two hybrids have been identified for potential commercialization in India and are being tested under on-farm conditions in 2014. The best varieties in advanced trials have up to 50 ppm iron and 40 ppm zinc. The first wave of biofortified sorghum is expected to be commercialized/released in 2015.

Capacity Building

In 2013, ICRISAT-Niamey's capacity was strengthened by installing and implementing X-ray fluorescence (XRF) machines for in-country mineral analysis of sorghum.

Regional Testing

ICRISAT-bred improved iron and zinc lines and hybrids are currently being tested at various locations in India, including at ICRISAT - Patancheru, Vasanthrao Naik Marathwada Krishi Vidyapeeth (VNMKV) - Parbhani, and Mahatma Phule Krishi Vidyapeeth (MPKV) - Rahuri in Maharashtra State. There is large genotype-by-environment (GxE) interaction for iron and zinc in sorghum. This makes it necessary to test genotypes at a large number of sites in more seasons in order to identify the most stable lines for commercialization. VNMKV-Parbhani is testing the improved line ICSR 14001 in state multi-location testing (10 locations) during 2014.

Highlights

- Promising varieties and hybrids have been identified for commercialization in India during 2015-16.

Challenges

- Because no in-country capacity for mineral analysis existed in Mali until 2013, obtaining iron and zinc concentration data has been delayed.

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2. Kumar, AA; et al. 2012. Genetic variability and character association in grain iron and zinc contents in sorghum germplasm accessions and commercial cultivars. *The European Journal of Plant Science and Biotechnology* 6(1): 66-70.
3. Kumar, AA; et al. 2013. Increasing grain Fe and Zn concentration in sorghum: progress and way forward. *Journal of SAT Agricultural* Research 11.
4. Ashok Kumar, Belum V.S. Reddy, B. Ramaiah, K.L. Sahrawat, Wolfgang H. Pfeiffer. 2013. Gene effects and heterosis for grain iron and zinc concentration in sorghum [Sorghum bicolor (L.) Moench]. Field Crops Research 146: 86–95.
5. A Ashok Kumar, Belum V S Reddy and KL Sahrawat. 2013. Biofortification for combating micronutrient malnutrition: Identification of commercial sorghum cultivars with high grain iron and zinc concentrations. Indian Journal of Dryland Agricultural Research and Development. 28(1): 95-100.

Measuring Provitamin A Content in Crops

Hernan Ceballos (CIAT) & Elizabeth Parkes (IITA)

In Mali, promising genotypes are being validated for iron and zinc concentrations. Progenies derived from high iron Guinea landrace donor parents and from the Diversified Dwarf Guinea Population are expected to provide novel diversity for micronutrient concentration, as well as achieve acceptable plant height.

Cassava is grown in areas where mineral and vitamin deficiencies are widespread, especially in Africa. A marginal nutrient status increases the risk of morbidity and mortality. Beta-carotene, the most potent and widespread form of provitamin A (1), is the predominant carotenoid in cassava, occurring as a mixture of *trans*- and *cis*-forms (2).

HarvestPlus supports breeding work to improve the nutritional quality of different crops, including increasing carotenoid content in cassava roots (3). Significant progress has been made over the past 10 years, including almost tripling the original concentration of carotenoids in cassava roots and gaining a better understanding of the impact of processing cassava roots on bioavailability (4,5).

In the past decade, the International Center for Tropical Agriculture (CIAT) has produced thousands of segregating progenies, which were evaluated in the field. Initially, data were analyzed simultaneously by high-performance liquid chromatography (HPLC) and spectrophotometry. Both measurements were taken for purposes of comparison, although HPLC data is more informative because it quantifies various types of carotenoids. HarvestPlus has recommended HPLC as a reference method and the spectrophotometer reading for Total Carotenoid Content (TCC). Regression analysis on more than 3,000 data points showed, as expected, a very close relationship between TCC as measured by HPLC and by spectrophotometry (regression coefficient 1.07 and R^2 value above 0.93). The protocol for carotenoids quantification is well established, and data has been found to be reliable and replicable (6).

A bottleneck in breeding emerged as the visual selection for root color method that was initially implemented became obsolete with the gradual development of large populations with deep yellow roots. Fresh roots are needed for quantification of carotenoids, as samples lose carotenoids in the process of drying and/or storage (7,8). Carotenoid content can be reliably quantified (through spectrophotometry or HPLC) but only for a limited number of samples per day. In most cases, breeding projects have a defined harvesting season because dry matter content (DMC) fluctuates depending on rainfall patterns; variation in DMC affects carotenoid quantification. Together, these limitations create a bottleneck in the number of samples that can be analyzed in each cycle of selection. An efficient system for pre-selection of the few samples to analyze is, therefore, highly desirable.

There are several approaches for pre-selection. At the International Center for Tropical Agriculture (CIAT), two prediction strategies were tested: near-infrared spectroscopy (NIRS) and Hunter color quantification with a chromameter. Predictions and carotenoid quantifications were based on fresh root samples. This is a key feature because freeze-drying equipment is not always available, and there is the potential for carotenoid losses through the processing of samples, as stated above.

Predictions based on NIRS were found to be highly satisfactory (9). The R^2 values for TCC were above 0.92 and for total beta-carotene (TBC) even better (0.93). In other words, more than 90 percent of the variation in the quantified levels of TCC or TBC can be predicted by the NIRS. Another advantage of NIRS is that analysis of a given sample can predict several other traits as well. DMC, for example, was very reliably predicted by NIRS ($R^2 = 0.96$) and improving predictions of cyanogenic glucosides content is underway (currently R^2 values are around 0.86). Drawbacks to NIRS include the cost of the equipment and the need to develop predictive equations.

The second pre-selection strategy that CIAT evaluated was the measurement of Hunter color with a chromameter. This is a simple and portable device that is considerably less expensive than the NIRS. Current predicting equations for TBC are very promising ($R^2 > 0.70$), independent of the levels of TBC actually quantified. For TCC, the chromameter has thus far produced less reliable results (9).

The International Institute of Tropical Agriculture (IITA) uses HPLC, spectrophotometer, and NIRS. IITA has also developed an alternative method - iCheck™ Carotene - introduced by BioAnalyt, which is used to quickly screen large populations especially at seedling and clonal stages of breeding. The test-kit consists of a portable photometer and ready-to-use reagent vials. This combination allows for cost-effective, simple, user-friendly, and rapid screening of large sample numbers, including at field locations with no electricity or refrigeration.

To use iCheck™, roots are harvested early in the morning and labeled, then washed and peeled. Samples are prepared, and 0.4 mL is taken from the slurry sample and injected into the reagent vial (iEx Carotene) included in the test kit. The vial is shaken and allowed to stand for a minimum of five minutes for carotenoids extraction before measurement is taken. Training conducted in March 2014 showed that the reading could be taken from five to 60 minutes during which time the carotenoids are stable in the vial. The vial is inserted into the device and measured. The device displays the result in milligram carotenoids per liter (mg/L). To get the concentration of TCC in cassava root, the result is multiplied by the dilution factor (total sample volume in water/sample weight).

The iCheck™ device needs to be handled carefully, but it is durable enough for field conditions. The device's calibration should be periodically checked with a solid photometric standard.

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Measuring Trace Micronutrient Levels in Crops

James Stangoulis & Georgia Guild (Flinders University)

Analytical tools to determine the levels of micronutrients in crops are an important aspect of plant breeding. Various techniques have been employed to quickly and accurately determine the levels of metals, particularly iron (Fe) and zinc (Zn), in plant material. These techniques include inductively coupled plasma optical emission spectroscopy (ICP-OES), atomic absorption spectroscopy (AAS), colorimetric staining, and more recently X-ray fluorescence spectroscopy (XRF).

ICP-OES and AAS

Analytical techniques such as ICP-OES and AAS have been well established and used for many years to determine metal concentrations (including micronutrients such as iron and zinc) in various samples. ICP-OES analysis is considered the "gold standard" due to the high accuracy and low limits of detection for micronutrient analysis in plant material. However, these techniques require several steps prior to analysis, including grinding the grain to form flour and digestion with acid, in order to extract the metals from the flour into a solution for analysis. Due to the sensitivity of these techniques (detection possible down to the µg/kg levels), it is vital to ensure the use of high purity reagents, specialized equipment, and highly trained staff to minimize potential contamination and ensure high quality analyses. The resulting solution containing the extracted metals from the flour is then analyzed with ICP-OES or AAS. This can identify which metals are present in the flour and the concentration of these metals in the original grain sample. These pieces of equipment are expensive to purchase and run, and samples will often need to be sent overseas for such analyses. This process is time consuming and expensive both in terms of analysis cost and time required for sample preparation, shipment, and analysis.

Colorimetric Approaches

Various alternatives to the above-mentioned analyses have been investigated to screen for crops with high levels of iron and zinc in an attempt to increase throughput and reduce analysis costs. One such alternative is the use of colorimetric approaches for staining grain in order to determine the concentration and localization of micronutrients in the seed. This method has shown a good correlation with ICP-OES analysis; however, the process is time consuming and not feasible when screening large numbers of samples.

X-ray Fluorescence Spectroscopy

More recently, XRF has been employed by HarvestPlus as an alternative means for micronutrient analysis and has been validated with two instruments, the Oxford Instruments XSupreme 8000 and the Bruker S2 Ranger (Figures 1 and 2). Unlike the previously discussed methods, XRF is able to analyze whole grain and flour samples without the need for digestion prior to analysis. This increases the sample throughput and reduces the pre-analysis preparation, consequently reducing the potential for sample contamination and analysis cost per sample. Additionally, XRF is much cheaper to purchase and run than ICP-OES and is easy to operate without the need for highly trained analysts, specialized facilities, or additional equipment.

Figure 1. Oxford Instruments XSupreme 8000

Figure 2. Bruker S2 Ranger

HarvestPlus and partners have developed methods for using XRF to screen various elements (iron, zinc, selenium, and phosphorus) in whole grain samples including rice, wheat, and pearl millet, and flour samples for larger grains such as beans and maize. The levels of detection are around 5 mg/kg for iron and zinc, which is ideally suited to micronutrient breeding programs. While XRF is less accurate than ICP analysis, its results show a strong correlation with the latter's analysis. This makes XRF ideal for screening large numbers of samples. XRF analysis can identify which samples have the highest levels of iron and zinc, and these samples can then be sent for further analysis (such as ICP-OES) for more accurate micronutrient determination. At this stage, HarvestPlus is unable to screen for aluminum or titanium, indicators of soil contamination known to affect iron levels. These elements can be analyzed with ICP, which further emphasizes the complementary nature of these two techniques in order to ensure high quality micronutrient analysis.

Cost / Time Effectiveness

Analysis of a single sample with XRF takes between 30 seconds and two minutes, depending on the crop. HarvestPlus has installed 18 of these instruments in various countries, and each instrument has been able to analyze 100–200 samples per day. These instruments have effectively paid for themselves, considering the thousands of samples analyzed each year with XRF rather than more expensive, alternative techniques.

Plant Breeding Basics

Torbert Rocheford, Megan Fenton, Brenda Owens (Purdue University); Christine Diepenbrock, Kathy Kandianis (Cornell University); & Tyler Tiede (University of Minnesota)

Plant breeding is the art and science of manipulating plants for the benefit of humans. Throughout history, humans have selected specimens for improved characteristics such as yield, quality, and flavor. The seeds of these selected plants formed the next year's crop, and repetition of this process over many generations resulted in improved, locally adapted populations that are often referred to as landraces.

Critical Need for Plant Breeding in Addressing Global Challenges

Plant breeding offers a mechanism for helping to address some of the world's most pressing and current concerns. One of the greatest challenges facing modern plant breeders is ensuring global food security in the face of a host of global and local obstacles. The current food supply is expected to be insufficient to support projected population growth, both in quantity and nutritional quality, thus necessitating plant breeding efforts that can increase production while using less land and fewer resources. Doing more with less will undoubtedly be a mantra of the plant breeding community moving forward.

Climate Change and Resource Limitations

Natural adaptation and selection are unable to keep up with the rate at which climates are changing. Resource limitations and environmental concerns are increasing global pressure to reduce agronomic inputs such as nitrogen, phosphorus, and water. Artificial selection within breeding programs for traits such as water use efficiency (WUE) and nitrogen use efficiency (NUE) may effectively respond to climate change and accelerate our efforts to feed current and future human populations and reduce agricultural inputs.

Monoculture and Improving Nutritional Quality

Low-diversity cropping systems (i.e., monocultures), along with the introduction of plant and insect species to non-native environments, have exacerbated the propagation of plant pathogens. Breeding to improve crops with natural adaptive capacity, as well as disease and pest resistance, can reduce threats associated with the systemic spread and propagation of plant pathogens. Furthermore, the growing focus on concurrent improvement of yield and nutritional quality of edible plant tissues emphasizes a critical role for well-trained plant breeders as human populations move from calorie-dense to nutrient-balanced diets.

Biomass Supply and Adapting to Cultural Practices

Plant biomass (grain and stover) is the substrate for not only food production but also fiber, feed, and fuel production. There is a growing need to balance the end use of plant biomass in a way that satisfies consumer needs. Plant breeders can play a role by developing new crops to fill a niche for various needs or by adding new priority traits for improvement within their existing programs. As another important consideration, cultural practices in agriculture vary widely across geographic regions. Different realities exist for smallholder farms that save seed from open-pollinated varieties or purchase from local markets or vendors versus large-scale, mechanized farming endeavors using hybrid crop varieties. The latter situation is more amenable to current breeding methodologies given the more stable performance of hybrid varieties, but such a focus will leave smallholder farmers in need.

Considerations for Improvement of Crops

Before beginning the breeding process, a trait must be defined, along with a system to measure phenotypes (a plant's performance for that trait). The diversity and range of phenotypic values must also be considered. For example, in biofortification the trait of interest is micronutrient content, and phenotypes are analyzed through various assays such as liquid chromatography and spectroscopy. The crossing type and propagation system must also be considered: is a crop propagated by seed or by tuber? Is the plant self-pollinating (such as beans) or does it depend on cross-pollination (such as maize)? How will success be defined, and what are the relevant measures of performance? What other traits are important for consumer acceptance (e.g., appearance, flavor, yield potential)? Should breeders seek adaptability to different environments or stability across all environments? How will genetic and phenotypic diversity be maintained to reduce disease susceptibility (as highly related lines risk being wiped out by the same strain), preserve plants' abilities to adapt, and maximize future gains from breeding?

Methodology

Breeders must consider the heritability of a trait—the proportion of phenotypic variance explained by genetic factors—and how this factors into selection of breeding methods and outcomes. Conventional breeding is phenotype-driven, meaning that selections are made based on visible traits. Typically, breeding populations are developed by crossing a small number of parental lines together, which allows the development of families that can be tracked and selected upon through the breeding process.

Breeding can use a strategy termed backcrossing, in which lines showing favorable phenotypes for the trait of interest are crossed back to one of the parents to regain some of the parent's superior phenotypes for other traits of interest. In the case of self-pollinating crops, the conventional goal is to make selections through multiple cycles of inbreeding until a stable, superior family or line is identified and released as a variety. For outcrossing species, the goal may be to inbreed families while testing their ability to complement families from other populations, such that the hybrid progeny resulting from a single cross is superior to the inbred counterparts of the parental populations.

Conventional breeding can easily become complicated and is further muddled by the population development procedure, which mixes different strands of DNA and swaps multiple genes at one time—the effects of which might not be immediately visible. Because even promising new lines must be tested over multiple generations, conventional breeding is a lengthy process. Marker-assisted selection, in which particular genes of interest are identified and selected upon, is by contrast genotype-driven. Using this method, scientists can analyze plant tissue from experimental crosses to see if it contains the genes of interest—cutting down on the time required to identify promising lines.

An extension of marker-assisted selection is genomic selection, in which genome-wide markers are each assigned particular weights based on their influence on a trait's phenotype across the whole plant population being studied. This methodology allows trait phenotypes and breeding values to be assigned to an individual plant based on its genotype alone, greatly reducing the cost and rigor of field trials and saving time by allowing individuals to be assessed early on during the breeding cycle.

The Future of Plant Breeding

Innovations such as high-throughput phenotyping and combining molecular genetics with crop models offer new frontiers for improving plant breeding science. High-throughput phenotyping offers an improved capacity to rapidly quantify phenotypic traits—especially whole-plant physiological traits (e.g., responses to low water status) in the field—providing valuable information on plant and environmental effects and their interactions, as well as a prognosis for plant performance. Advances in methods and techniques for understanding epigenomics, or the regulatory patterns underlying gene expression, are allowing breeders to determine and use key traits enabling plants to adapt to their environment—for example, flowering time—to better survive environmental stressors. Also on the horizon are promising technologies that can target mutations and insertions to specific portions of the genome, enabling more precise breeding to support the needs of a growing and changing world.

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Agronomic Biofortification

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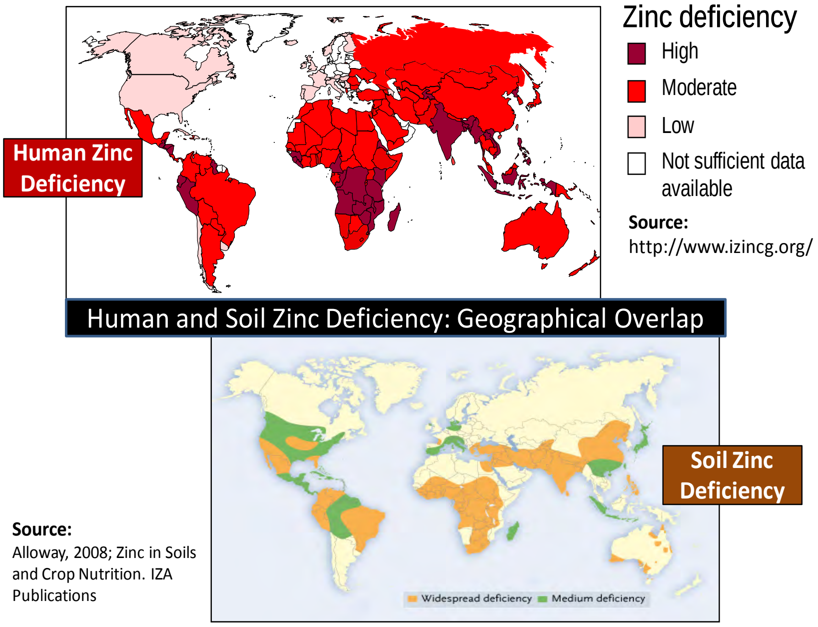
Agronomic biofortification of food crops is a strategy, along with breeding/genetic engineering, for increasing micronutrient concentrations to reduce dietary deficiencies. Today, increasing micronutrient concentrations of stable food crops, especially in cereal grains, represents an important humanitarian challenge and a high-priority research area. Soil and foliar application of micronutrient fertilizer can be used for several different mineral micronutrients to varying effectiveness. Agronomic biofortification, especially in the case of foliar application, is highly effective for zinc and selenium, while also effective for iodine and cobalt. As an effective strategy for reducing micronutrient deficiency, zinc provides one of the best and quickest avenues for agronomic biofortification, particularly within cereal crops.

Zinc Deficiency in Human Populations and Crop Production

Zinc deficiency is a well-documented global micronutrient deficiency problem both in human populations and in crop production. It is estimated that about 50 percent of the cereal-cultivated soils globally are deficient in plant-available zinc, leading to reductions in crop production and also nutritional quality of the harvested grains (1,2). Since cereals are inherently low in zinc, growing them on such potentially zinc-deficient soils further reduces grain zinc and thus the dietary intake of zinc when eaten. In many developing countries, cereals represent the major source of daily caloric intake. Dietary zinc deficiency is associated with severe consequences in human health, including impairments in brain function and development, weakness of the immune system to deadly infectious diseases, and delays in physical development. As shown below, it is not surprising that the well-known zinc deficiency problem in humans occurs predominantly in the countries/regions where soils are low in available zinc, and cereals are a major staple.

Figure 1. Zinc Deficiency in Humans and Soil



Source: http://www.izincg.org/

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HarvestPlus Zinc Fertilizer Project

The HarvestPlus Zinc Fertilizer Project, called HarvestZinc, is exploring the potential of various zinc-containing fertilizers for increasing zinc concentration in cereal grains and improving yield in target countries such as India, China, Pakistan, Thailand, Laos, Turkey, Zambia, Mozambique, and Brazil (see www.harvestzinc.org). The results obtained under the HarvestZinc project demonstrate that foliar or combined soil plus foliar application of zinc fertilizers under field conditions is highly effective in increasing grain, especially in wheat. Zinc-enriched grains are also of great importance for crop productivity, resulting in better seedling vigor, denser stands, and higher stress tolerance in potentially zinc-deficient soils. Agronomic biofortification is essential for keeping sufficient amounts of available zinc in soil solution (by soil zinc applications) and in leaf tissue (by foliar zinc applications), which greatly contributes to the maintenance of adequate root zinc uptake. It also assists with transport of zinc from leaf tissue to the seeds during their reproductive growth stage. This approach is also required for ensuring and maximizing the success of biofortified food crops that are bred with higher levels of zinc.

Increasing grain zinc concentrations through foliar zinc applications is similar to increasing zinc concentrations in other parts of the grain such as the endosperm, which is the most commonly eaten part of wheat grain. Since phytate (an antinutrient that inhibits zinc bioavailability in humans) in the wheat grain endosperm is very low, or not detectable, the increases in zinc concentration in the endosperm (up to 3-fold) by foliar zinc spraying is important for human nutrition, as it could result in higher zinc bioavailability.

Additional results from the foliar zinc spray project include:

- Among wheat, rice, and maize, wheat has been found to be the most promising cereal crop for increasing zinc in grains through foliar zinc fertilization.
- Foliar zinc fertilizers can be sprayed on leaves together with fungicides/insecticides. When tested in different countries, there was no observed adverse effect of those pesticides on leaf zinc penetration and seed/grain zinc deposition.
- Increasing nitrogen fertilization of plants very positively affected shoot translocation and grain deposition of foliarly applied zinc.
- Among the zinc forms tested for foliar zinc application (ZnO, $ZnCl_2$, ZnEDTA, nano-sized ZnO particles, and ZnSO4), ZnCl2 and ZnSO4 gave the best result while ZnO and nano-sized ZnO particles were less effective in increasing grain zinc.
- Foliar spray solution pH and use of some adjuvants markedly affect the agronomic effectiveness of foliar zinc fertilizers. Reducing pH from 8.3 to 5 increased grain zinc concentration up to 60–70 percent.

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Transgenic Biofortified Crops

Joe Tohme (CIAT-HarvestPlus) & Peter Beyer (University of Freiburg, Germany)

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 (e.g., 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 ensure 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.

To attain higher levels of provitamin A, zinc, and iron content in crops where genetic variation for these traits has not been identified, HarvestPlus, its partners, and other organizations have explored transgenic approaches, discussed below.

Golden and High-Iron Rice

Golden Rice was first developed at the Swiss Federal Institute of Technology and the University of Freiburg, Germany. The inventors donated the technology for public sector research and development and farmers’ use, free of charge, in developing countries. This effort was assisted by Syngenta, which arranged, for humanitarian purposes, royalty-free access to intellectual property for a number of key technologies used in Golden Rice held by several biotechnology companies. These arrangements allow the International Rice Research Institute (IRRI) and others to develop Golden Rice on a not-for-profit basis. In parallel, Golden Rice product development was furthered by Syngenta as part of its then-commercial pipeline. Transgenic events with higher levels of provitamin A, up to 37 ppm in a U.S. variety (GR2 events), were produced and then donated for use by the Golden Rice Network when Syngenta decided not to pursue the trait as a commercial product (1). The development of Golden Rice is currently coordinated by IRRI in collaboration with national rice research institutes such as PhilRice (Philippines), Bangladesh Rice Research Institute (BRRI), and Indonesian Centre for Rice Research (ICRR). Starting in 2006, the GR2 events were backcrossed into varieties for these countries. 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 3.8:1 and 2:1 (2,3). Golden Rice will be required to pass biosafety tests prior to release. An efficacy trial, evaluated by Helen Keller International, is planned in the Philippines after biosafety approval is granted. For additional information, see www.goldenrice.org and http://irri.org/golden-rice.

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. This variety translocates iron to accumulate in the endosperm, where it is unlikely to be bound by phytic acid and, therefore, likely to be bioavailable (4). The University of Melbourne has produced a number of transformants of Nipponbare carrying the rice nicotianamine synthase (NAS2) over expression genetic constructs, suggesting, at screen-house level, the ability to reach target levels for iron and zinc. Teams at IRRI have produced several thousand transformants of IR64 and IR69428 that carry the soybean or rice ferritin and NAS2 over expression genetic constructs and, in the field, demonstrate the target level for iron and surpass that for zinc. Achieving the iron and now higher zinc levels in the field requires both a ferritin and NAS gene to be expressed correctly. The project at IRRI is now moving beyond proof of concept to product development for high-iron and high-zinc, highly adapted rice genotypes. Bioavailability trials are expected to begin next year, and release is projected for about 2022 in Bangladesh.

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 (5). 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 (6). For additional information, see BioCassava Plus at http://www.danforthcenter.org.

Vitamin A and Iron Bananas

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 (7). Provitamin A bananas are expected to be released in 2019. A human bioavailability study using transgenic provitamin A banana began in late 2013. High-iron bananas are not yet ready for use in human trials. For additional information, see Banana21 at http://www.banana21.org/index.html.

Iron Wheat

Efforts to increase iron concentrations in wheat by conventional breeding have not been successful, and there are currently no iron-biofortified wheat varieties available for farmers. Whole wheat grain contains approximately 30 ppm iron, of which only 5 percent is estimated to be bioavailable. It is estimated that wheat requires an additional 22 ppm iron in the whole wheat grain, for a total concentration of 52 ppm iron, to adequately biofortify a wheat-based diet with iron.

The University of Melbourne is employing the approach that has proven highly effective in rice, using NAS to increase iron concentrations in wheat and produce biofortified wheat varieties with 52 ppm iron in whole grain. The project places strong emphasis on multi-location field trials of wheat plants transformed with a rice nicotianamine synthase gene (OsNAS2) under regulatory control of the maize ubiquitin promoter, Ubi1, to provide proof of concept of the transgenics approach in wheat. Additionally, selectable marker-free transgenic populations will be developed and evaluated in commercially important wheat backgrounds.

The John Innes Center is investigating several independent strategies to increase iron concentration and bioavailability in wheat grains through transgenic means. The approaches will target distinct stages and tissues including uptake from the soil, remobilization from vegetative tissue during grain filling, and accumulation within grains to enhance total iron in the grain (8).

Challenges

Regulatory concerns are often the biggest sticking point for the rollout and adoption of transgenic crops and continue to be a significant barrier (9).

Recommendations

Use conventional breeding where the genetic variability for the nutritional trait is sufficiently large and breeding is feasible. Apply recombinant transgenic technologies when this is not available.

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Prevalence and Consequences of Mineral and Vitamin Deficiencies and Interventions to Reduce Them

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Prevalence

As a result of consistently consuming monotonous diets based predominantly on staple crops such as maize, wheat, rice, cassava, etc., that provide large amounts of energy but relatively low amounts of essential vitamins and minerals, people develop nutritional deficiencies. These deficiencies render them unable to produce the bioactive molecules needed for proper physical, mental, and cognitive development and optimal income-generating work. From a social perspective, populations affected by vitamin and mineral deficiency at levels that affect public health cannot achieve their economic potential. Roughly more than one-third of the world's population is at risk of one or more micronutrient deficiencies. Iron deficiency is the most common micronutrient deficiency in the world. However, global data for iron deficiency does not exist, and anemia is used as an indirect indicator. Globally, the most common trace element deficiencies in order of prevalence are iron (~1. 6 billion affected by anemia) (1), iodine (~2. 0 billion) (2), and zinc (~1. 5 billion) (3). The most widely prevalent vitamin deficiencies of public health significance are vitamin A with 190 million preschool children and 19 million pregnant women at risk (4), folate, and B12.

The estimated regional prevalence of three principal micronutrient deficiencies is described in the table below. It should be noted, however, that in these populations, the poorest bear the brunt of preventable mental disability and diminished physical performance, maternal and fetal-child deaths, and other long-term negative effects that constrain socioeconomic development. The lack of each nutrient deteriorates human health independently, but their combination undermines the potential of human capital at both the individual and collective levels and is difficult to measure accurately.

Table 1. Regional Prevalence of Micronutrient Deficiencies

WHO Region	Vitamin A Deficiency[1]		Anemia (Proxy Indicator of Iron Deficiency)[2]			Iodine Deficiency[3]
	Preschool-age children	Pregnant women	Preschool-age children	Pregnant women	Non-pregnant women	School-age children
Africa	44.4	13.5	67.6	57.1	47.5	40.8
Americas	15.6	2	29.3	24.1	17.8	10.6
Europe	19.7	11.6	21.7	25.1	19	52.4
Eastern Mediterranean	20.4	16.1	46.7	44.2	32.4	48.8
South-East Asia	49.9	17.3	65.5	48.2	45.7	30.3
Western Pacific	12.9	21.5	23.1	30.7	21.5	22.7
Global	33.3	15.3	47.4	41.8	30.2	31.5

[1] *Defined as serum retinol <0.7 umol/L.* Global Prevalence of Vitamin A deficiency in populations at risk 1995-2005; WHO Global Database on Vitamin A Deficiency

[2] *Defined as haemoglobin <110 g/L (pre-school children and pregnant women) and <120 g/L (non-pregnant women*). Worldwide Prevalence of Anaemia 1993-2005, World Health Organization, 2008

[3] *Defined as urinary iodine < 100 ug/L.* Iodine Deficiency in 2007: Global progress since 2003; World Health Organization, 2008

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Consequences

The adverse sequelae of these deficiencies are profound and include premature death, poor health, blindness, stunting, mental retardation, learning disabilities, and low work capacity. The negative effects of micronutrient deficiencies damage human capital and national economic development, particularly in developing countries.

The health issues associated with the most common micronutrient deficiencies (i.e., vitamin A, iron, and zinc) are:

- **Vitamin A:** Blindness, impaired immune system function, abnormal fetal development, increased child mortality, and increased maternal mortality.
- **Iron:** Iron deficiency anemia, reduced cognitive capability, reduced physical capacity and productivity, increased maternal mortality, complications with childbirth, and increased infant mortality.
- **Zinc:** Decreased resistance to infectious diseases, stunting and impaired growth in children, and increased infant and child mortality.
- **Iodine:** Impaired mental development and brain damage, lower birth weight, and increased infant mortality.

Interventions

The consequences of micronutrient deficiencies are undoubtedly tragic for individuals and families. The other dimension of this tragedy is the fact that most of these consequences can be prevented by currently available, cost-effective interventions. Traditionally, approaches to prevent micronutrient malnutrition have been grouped into medicinal (supplementation), food-based (food fortification, homestead food production, biofortification), nutrition education, and public health interventions (environmental sanitation, deworming, malaria control, etc.). More pragmatically, large-scale micronutrient interventions can be classified, based on the level of scientific evidence underpinning them, as follows (5):

1) **Interventions with strong evidence of effective implementation and impact at large scale** (i.e., preschool vitamin A supplementation, mass fortification of salt with iodine, sugar fortified with vitamin A, and wheat flour fortified with folic acid);

2) **Micronutrient interventions needing further confirmation of implementation effectiveness and impact** (i.e., maternal iron and folic acid supplementation and mass iron fortification programs); and,

3) **Emerging micronutrient interventions that hold promise but lack implementation experience at large scale** (i.e., home-based fortification and biofortification).

There is no single magic bullet for populations living in poverty, hunger, social marginalization, unsanitary environments, and with low quality/coverage of health services. In practice, distinct combinations of available evidence-based interventions are necessary to address the problem effectively and sustainably in different socioeconomic and cultural contexts. Supplementation should be a short-term intervention for individuals and population groups during particularly high-risk phases of the life cycle (for example, iron for pregnant women and iron and vitamin A for children under 2 years of age). Enhancing the vitamin and mineral content of staple foods and widely consumed condiments will improve a given population's general micronutrient status and provide the necessary reserves to meet normal day-to-day requirements and prevent deficiency when more is needed. The best and lasting solution to eliminating undernutrition as a public health problem in developing countries is to permanently consume a range of nutrient-rich staple and non-staple foods—food and nutrition security for all. Until that is achieved, biofortification of staple foods has the potential to lift the micronutrient intake for millions of people at no additional cost to consumers.

Micronutrient deficiency control and prevention programs should be tailored to existing country capabilities, and plans for sustained intervention should use multiple strategies (such as supplementation, fortification, food-based approaches, and public health measures) and address multiple micronutrient deficiencies. Because the nutrition status of all populations is in flux and groups are on a continuum of nutritional risk (from severe malnutrition, through several stages of nutrient adequacy, to nutrient overload and toxicity at the opposite end), micronutrient programs should aim to move at-risk groups from a phase of public health risk to states of sufficiency and health.

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PROGRESS BRIEF #19

NUTRITION & IMPACT

Efficacy and Other Nutrition Evidence for Vitamin A Crops

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Biofortified crops as sources of vitamin A contain provitamin A carotenoids, which are a precursor of vitamin A, similar to those found in some plant foods like carrots, mangos, and papaya. Two important factors to consider in the provitamin A crops are retention and bioavailability. The bioavailability of the most common plant provitamin A (beta-carotene) varies widely in relation to the food crop, genotype, cooking method, individual genetic factors, and consumption of fat with the meal. Bioconversion of dietary beta-carotene to retinol across plant foods ranges from 3 to 28:1 by weight (1). Food carotenoid degradation displays exponential decay patterns during storage and varies with the food matrix, crop genotype, and form of storage. It increases directly with time and temperature during storage as well as with exposure to high temperature, light, acids, and oxygen during processing. Because the bioconversion of provitamin A carotenoids to vitamin A is homeostatically regulated*,* excess toxic accumulation of retinol is prevented. Described below are the studies on retention, bioavailability and the efficacy trials conducted to date on biofortified provitamin A crops.

Biofortified Sweet Potato (*Ipomoea batatas*)

Orange sweet potato (OSP) growing in Africa south of the Sahara have high levels of *beta*-carotene (100–1600 *µ*g retinol activity equivalent (RAE)/100 g fresh weight) and are well accepted by young children (2,3).

Retention: Boiling sweet potato for about 30 minutes retained 80–90 percent of beta-carotene (3) while steaming for the same amount of time resulted in 70–75 percent beta-carotene retention (4).

Bioavailability: A study in Bangladeshi men who consumed a high beta-carotene sweet potato reported a bioconversion of 13:1 bioequivalence between *beta*-carotene and retinol (5).

Efficacy trials: An efficacy study conducted in South Africa with children aged 5-10 showed that the 3,4-didehydroretinol to retinol ratio (DR:R) was significantly greater in the OSP group than in a control white sweet potato group after 53 days of intervention ($p=0.0203$) (6). A smaller placebo-controlled randomized trial in Bangladesh with 14 adult men demonstrated that an additional 750 µg RAE/day as boiled canned OSP puree produced higher final serum retinol and beta-carotene concentrations ($p<0.03$) (7).

Effectiveness: The introduction and promotion of OSP was assessed in Mozambique over four growing seasons and demonstrated that serum retinol increased significantly at endline for children in the OSP intervention group ($p<0.001$) (8). The OSP intervention reached 24,000 households in Uganda and Mozambique from 2006–2009 with adoption rates greater than 60 percent above control communities (9). Introduction of OSP in rural Uganda resulted in increased vitamin A intakes among children and women and improved vitamin A status among children by decreasing the prevalence of low serum retinol (<1.05 mmol/L) by 9 percentage points (10).

Preliminary adjusted figures for the marginal cost per beneficiary were $5–8 and $6–10 in Uganda and Mozambique, respectively. Each DALY (disability-adjusted life year) saved would cost US$15–24 (11).

Biofortified Cassava (*Manihot esculenta*)

Cassava is an important food security crop for populations deriving sustenance from infertile soils. Total carotenoid content in cassava is 7.99 µg/g (range: 2.87–12.95 µg/g fresh weight), of which approximately 70 percent is beta-carotene (12).

Retention: The most common cassava processing methods in developing countries (i.e., boiled, *gari*, *fufu*, fermented and unfermented flour) resulted in losses of *beta*-carotene ranging from 30 percent (boiled) to 70 percent (*gari*) (12).

Bioavailability: Bioconversion studies have shown a 5:1 bioequivalence between *beta*-carotene and retinol (13).

Efficacy trial: An efficacy study conducted in Eastern Kenya with children aged 5-13 showed an increase in serum retinol of 0.038 µmol/L ($P<0.05$) and in *beta*-carotene of 43.78 µmol/L ($P<0.05$) in the yellow cassava versus the control group (14).

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Biofortified Maize (*Zea mays*)

Maize provides an estimated 15 percent of the world's protein and 20 percent of the world's calories. Maize is, therefore, considered a staple food for more than 200 million people (15).

Retention: A retention study was conducted in Zambia and showed provitamin A losses of 50 percent in four genotypes after 15 days of storage in ambient conditions, after which the provitamin A content stabilized for six months of storage (16). However, retention of 90–100 percent was observed with milled products, *nshima*, and porridge (16). *Beta*-carotene degradation associated with traditional African household processing methods was approximately 25 percent for fermented/unfermented porridges (17).

Bioavailability: Bioconversion studies have shown a 3:1 (18) and 7:1 (19) bioequivalence between *beta*-carotene and retinol.

Efficacy trials: An efficacy study conducted in the Eastern province of Zambia with children aged 5-7 showed that the total body stores of vitamin A in the children in the orange maize group increased significantly compared to those in the negative vitamin A control group; data were adjusted for infection (*unpublished*). Preliminary results suggest improvement of total body stores of vitamin A and visual adaptation to darkness even within a context of marginal-to-adequate baseline vitamin A status among study subjects. The beta-carotene in maize is an efficacious source of vitamin A when consumed as a staple crop.

Highlights

Nutrition studies have demonstrated that OSP is an efficacious intervention; promising results are available on efficacy of maize and cassava.

Challenges

The yellow/orange color of vitamin A crops may be a barrier to consumer acceptance in some cases. White maize is preferred over yellow/orange maize in Zambia; this is not the case with yellow cassava in Nigeria, where yellow *gari* (made yellow with red palm oil) can be found in the market.

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Efficacy and Other Nutrition Evidence for Iron Crops

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After plant breeders have successfully developed varieties of selected staple foods with increased iron content, the edible portion of the food crops must be tested for a number of qualities before the crops can be considered for introduction into the food supply. Efficacy, or the demonstration of a significant impact on the nutritional status of human subjects who consume the staple food under controlled experimental conditions, must be demonstrated. To date, three iron-biofortified staple foods – beans in Mexico and Rwanda, rice in the Philippines, and pearl millet in India – have been tested for efficacy in populations that consume those staple foods as a major component of their normal diet. A systematic evaluation of all of the iron biofortification efficacy studies follows.

Methodology for Establishing Efficacy

All four efficacy studies previously completed have employed a randomized, controlled experimental study design. The iron-biofortified or similar control food was prepared to local tastes. Human research subjects consumed the foods for between 100 and 270 days depending on the study. Meal consumption was monitored that allowed observation of dietary iron intakes from the total diet as well as precise quantification of the iron consumed from the biofortified staple food of interest. All studies assessed iron status (blood hemoglobin, serum ferritin, soluble transferrin receptor, and total body iron) at baseline and again at the end of the feeding trial, and analysis focused on the feeding group difference in the change in iron status between baseline and endline.

Proof of Concept and Nutrition Evidence

The first efficacy study demonstrated “proof of concept” when consumption of iron-biofortified rice for nine months resulted in an increase in serum ferritin and total body iron in non-anemic Filipina religious sisters (Table 1). Biofortified pearl millet was evaluated in secondary school children from western Maharashtra, India. A significant improvement in serum ferritin and total body iron was observed in iron-deficient adolescent boys and girls after consuming pearl millet flat bread twice daily for four months. The prevalence of iron deficiency was reduced significantly in the high iron group, and for those children who were iron deficient at baseline, significantly more (64 percent) resolved their deficiency by six months. This study demonstrated that iron-biofortified pearl millet is effective in improving iron status in children.

Biofortified beans were tested for efficacy in two different populations. Mexican primary school children were observed to have improved transferrin receptor levels after consuming biofortified black beans for 105 days; however, the acute phase protein, serum ferritin, did not improve primarily because of high levels of infection in this population. In Rwanda, iron-depleted university women showed a significant increase in hemoglobin and total body iron after consuming biofortified beans for four-and-a-half months.

Variance in Iron Concentration

Iron concentration in the consumed portion of the staple food was lowest in rice (9.8 µg/kg) compared to pearl millet and beans in the other three trials (86–97 µg/kg). The combination of relatively higher iron concentration and the large quantities of staple food consumption of Rwandan beans and Indian pearl millet contributed 68 percent and 123 percent, respectively, to meeting the estimated average requirement (EAR) for absorbed iron in these populations.

Potential Factors Influencing Variation

Iron biofortification of select staple food crops has been shown to be efficacious when feeding trials followed specified guidelines to ensure: (1) adequate iron concentration difference existed between high-iron and control foods used in the study; (2) subjects were iron deficient at baseline; (3) sufficient consumption of the staple food was documented; (4) adequate time elapsed to see a response; and, (5) appropriate biomarkers of iron status were used. Other factors can influence demonstration of efficacy. They include low iron bioavailability in the staple food due to inhibitors of absorption in the diet, other non-staple food sources of iron in the diet, and the health and nutritional status of the study subjects.

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Improving Future Efficacy Studies

The strength of the findings for each study and across all four studies will be evaluated relative to the iron status of the study population at baseline, their inflammation status, dietary components that might affect absorption, dose of absorbable iron consumed, subject compliance, and length of feeding time. These results will be discussed in terms of how to improve future efficacy studies for iron-biofortified crops.

Continued Research and Challenges

Further analysis will focus on the effect of improving iron status on physical and cognitive performance in order to assess costs versus benefits of iron biofortification. In addition, HarvestPlus and its partners will be analyzing the ability of iron-biofortified beans and pearl millet to reduce the prevalence of iron deficiency in populations. Challenges remain to breed for crops with higher levels of biologically available iron that approach the levels seen in the Rwanda and India studies.

Table 1: Comparison of Results from Four Iron Biofortification Efficacy Studies[1]

Staple Food Crop (Location)	**Rice (Philippines)[2]**		**Beans (Rwanda)**		**Beans (Mexico)**		**Pearl Millet (India)**	
Subjects	**Adult Females**		**Adult Females**		**Children (M+F)**		**Youth (M+F)**	
Experimental group	**High Iron**	**Control**	**High Iron**	**Control**	**High Iron**	**Control**	**High Iron**	**Control**
Number of subjects	69	69	116	118	269	166	99	98
Hemoglobin (g/dL)	0.11	0.09	0.3*	-0.10	0.00	0.60	-0.14	-0.15
Ferritin (µg/L)	1.1*	-4.27	4.04	2.65	3.20	5.20	5.7*	1.2
Transferrin receptor (mg/L)	0.35	-0.15	-0.26	0.09	-0.10*	0.10	0.19	0.21
Body Iron (mg/kg)	0.63*	-0.25	1.36*	0.43				0.02
Sample Description	Non-anemic (Hb>12g/dL) at baseline		Low ferritin (<20 µg/L) at baseline		Low morbidity/ low inflammation schools		Low ferritin (<15µg/L) at baseline	

[1] Values reflect change in iron status indicator from baseline to endline.

[2] See Haas et al, *J Nutr* 135:2823-2830; 2005.

*Significant difference between high- and low-iron groups, Wilcoxon 2-group comparison test, p<0.05

Table 2: Iron Intakes from Biofortified Staple Food

Staple Food Crop (Location)	**Rice (Philippines)**		**Beans (Rwanda)**		**Beans (Mexico)**		**Pearl Millet (India)**	
Experimental Group	**High Iron**	**Control**	**High Iron**	**Control**	**High Iron**	**Control**	**High Iron**	**Control**
Iron content								
Concentration (µg/kg-dry)	9.8	1.9	86	51	95	55	87	30
Intake from Staple (mg/d)	1.8	0.4	13.5	8.0	4.7	2.6	17.6	5.7
Percent of Total Dietary	18	5	64	46	26	19	90	81
Iron Intake Relative to Requirements								
Percent Iron Absorption[1]	7.3	7.3	7.3	9.2	5.0	5.0	7.4	7.5
Absorbable Iron (µg/d)	134	30	986	737	233	132	1300	428
EAR for Iron (µg/d)[2]	1460		1460		800		1060	
Percent EAR from Staple	9	2	68	51	29	17	123	40

[1] Iron absorption estimates: Philippines rice from by Beard et al. (J Nutr 137:1741;2007); Rwanda beans from Petri et al. (*J Nutr 143:1219;* 2013); Mexico beans estimated from algorithm of Hallberg and Hulthen (*AJCN* 71:147;2000); pearl millet from Cercamondi et al. (*J Nutr* 143:1376; 2013)

[2] EAR = Estimated Average Requirement (from Institute of Medicine, Food and Nutrition Board, *Dietary Reference Intakes-DRI, US National Research Council, 2001.*

PROGRESS BRIEF #21

NUTRITION & IMPACT

Efficacy and Other Nutrition Evidence for Zinc Crops

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After plant breeders have successfully developed varieties of selected staple foods to increase the zinc (Zn) content, the foods must be tested for a number of qualities before they can be considered for introduction into the food supply. Efficacy, or the demonstration of a significant impact on the nutritional status of human subjects who consume the staple food under controlled experimental conditions, must be demonstrated. Efficacy studies are planned for biofortified wheat and rice in 2014 and 2015.

Zinc-biofortified Wheat

Rosado et al. carried out a trial comparing zinc absorption from biofortified versus conventional wheat as 95 and 80 percent extraction flours (1). Adult women consumed 300 grams of each type of the high- and low-extraction flours as tortillas for two consecutive days using either biofortified (41 mg Zn/g) or control (24 mg Zn/g) wheat. Mean (± SD) total zinc absorption from biofortified wheat was 2.1 ± 0.7 mg/d and 2.0 ± 0.4 mg/d for 95 and 80 percent extraction, respectively, each of which was 0.5 mg/d higher than the corresponding control ($p<0.05$). The authors concluded that "potentially valuable increases in zinc absorption can be achieved from biofortification of wheat with zinc." In 2013, a zinc absorption trial was conducted in Switzerland with the same type of wheat flour produced for future efficacy trials. Results will be published in 2014.

Two efficacy trials using biofortified wheat will be conducted in India in 2014, one among school children by the Swiss Federal Institute of Technology (ETH-Zurich) and another among preschoolers and their mothers by Cornell University.

Zinc-biofortified Rice

A 2010 zinc bioavailability pilot trial, designed to estimate the amount of zinc absorbed from zinc rice and compare that with absorption from conventional rice using the triple stable isotope tracer ratio technique, did not produce detectable differences in absorbed zinc (2). High phytate and lower-than-expected zinc in the biofortified zinc variety resulted in no significant difference in absorption between groups. The study was redesigned with a prospective biofortified variety containing more than 10 ppm zinc and comparable phytate concentration with that of the control. In September 2013, rice varieties with a 12 ppm differential were selected, and the absorption trial planned accordingly. The study is expected to be completed by September 2014. An efficacy study will follow.

Other indirect evidence is available for zinc. Although pearl millet is considered by HarvestPlus to be primarily an iron-biofortified crop, it provides some useful evidence regarding zinc biofortification. In their recent study comparing biofortified and conventional pearl millet in young children in India, Kodkany et al. showed that the quantities of zinc absorbed from test and control groups were 0.95 ± 0.47 mg/d and 0.67 ± 0.24 mg/d, respectively ($p=0.03$) (3). The authors concluded that the quantities absorbed from the biofortified pearl millet were more than adequate to meet the physiological requirements for zinc in children aged 2 years. Those results contribute to the accumulating evidence that zinc biofortification can be successful.

Results from ongoing or planned controlled efficacy studies using zinc-biofortified foods will provide further proof of concept. Lowering phytate levels could increase the bioavailability and potential for efficacy and effectiveness of zinc crops.

Challenges

HarvestPlus convened an expert consultation in 2012 to review the normative physiological zinc requirements, which concluded that the reference value for adult women used to set the original zinc breeding targets (1.86 mg/d) had to be revised up to 2.5–2.9 mg/d to conform to current evidence on the subject. This increase in physiological requirements resulted in the breeding target for wheat and rice being raised to +12 ppm above baseline, which will contribute around 20 percent of the zinc EAR for a woman of childbearing age.

For zinc-biofortified crops, it is recommended that lower phytate levels also be included in the traits pursued by plant breeders in order to increase bioavailability and biological impact in vulnerable population groups.

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Biofortification Prioritization Index

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Biofortification could prove to be a cost-effective and sustainable strategy for alleviating micronutrient deficiencies in rural areas of developing countries. The majority of households living in such areas rely on diets composed of staple foods, while their access to food supplements and commercially marketed fortified foods is limited. Research evidence to date suggests that biofortification is an efficacious and cost-effective public health intervention.

As evidence builds in favor of biofortification, stakeholders are increasingly interested in investing in this intervention to reduce micronutrient deficiency. These stakeholders need evidence-based information on where to target specific biofortified crops to achieve the highest nutrition and, hence, health impacts cost-effectively. The Biofortification Prioritization Index (BPI) contributes to closing this information gap by generating country-crop-micronutrient specific indices, ranking countries globally and within regions (Africa, Asia, and Latin America and the Caribbean [LAC]) according to their suitability for investment in biofortification interventions.

The BPI is calculated using secondary country-level data compiled from various sources, including the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), and the United States Department of Agriculture (USDA). Similar to the Human Development Index (HDI) and Global Hunger Index (GHI), a heuristic approach is used to generate the BPI using three sub-indices: one each for the consumption and production of the crop and one for the micronutrient deficiency. The consumption sub-index measures the intensity of consumption of the specific crop adjusted for the share of the crop's total national consumption that is imported. The production sub-index measures the national intensity of production by considering both per capita area harvested and national land area allocation to the crop, while adjusting for exports. Finally, the micronutrient deficiency sub-index measures the extent of deficiency of the micronutrient in question within the country. These consumption, production, and micronutrient deficiency sub-indices are then combined using a geometric mean to create the overall BPI.

The unweighted BPI is calculated by giving each country equal weights. However two "weighted" versions of the BPI were also generated that take into account either the country's (1) share of the target population (children aged 6–59 months and women of childbearing age) in the global target population or (2) the share of cultivated land area for a specific crop in the global cultivated land area for that crop. Both weights implicitly consider cost aspects, since fixed investments in biofortification for a given country can either benefit more people or be planted on more land. The weighted BPIs are meant to serve as *complementary* tools for specific stakeholders. The population-weighted BPI could be used by stakeholders whose mandate is to reach as many beneficiaries as possible, whereas the area-weighted BPI could benefit those whose aim is to maximize area allocated to biofortified crops. It is highly recommended that the weighted BPIs not be considered in isolation when making investment decisions, but rather in tandem with the unweighted BPI.

The table below presents the top 15 global rankings for the unweighted BPI. The results show that among the 127 countries included in the analysis, African countries rank highest for vitamin A biofortified crops, including maize, cassava, and sweet potato, and Asian countries rank highest for zinc biofortified cereals, including wheat and rice. For rice, Africa also offers some suitable candidates for generating high levels of impact. For iron-biofortified beans, several countries in Africa and some in LAC have high potential. Regarding iron biofortified pearl millet, both Africa (especially West Africa) and South Asia constitute suitable candidate sites for investment. Several of the findings are in line with HarvestPlus' currently implemented and planned biofortification interventions, while others suggest new avenues for expansion. Although results of the weighted BPI are not presented here, comparison of the unweighted and the weighted BPIs suggest that the three kinds of BPI could prove useful for stakeholders seeking to achieve different objectives.

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Table 1. Global BPI Ranking of Top 15 Countries, by Crop

Global Rank	Cassava (Vitamin A)	Maize (Vitamin A)	Sweet Potato (Vitamin A)	Beans (Iron)	Pearl Millet (Iron)	Rice (Zinc)	Wheat (Zinc)
1	Mozambique	Malawi	Angola	Rwanda	Niger	Cambodia	Tajikistan
2	Angola	Benin	Burundi	Benin	Gambia	Bangladesh	Turkmenistan
3	Ghana	Zambia	Uganda	Tanzania	Burkina Faso	Laos	Azerbaijan
4	Liberia	Kenya	Mozambique	Burundi	Chad	Myanmar	Afghanistan
5	Benin	Mozambique	Rwanda	Myanmar	Senegal	Viet Nam	Pakistan
6	Central African Republic	Angola	Tanzania	Togo	Nigeria	Indonesia	Kazakhstan
7	Democratic Rep. Congo	Burkina Faso	Sierra Leone	Haiti	Namibia	Sierra Leone	Uzbekistan
8	Sierra Leone	Zimbabwe	Madagascar	Uganda	Guinea-Bissau	Madagascar	Turkey
9	Côte d'Ivoire	Timor-Leste	Guinea	Angola	Uganda	Sri Lanka	India
10	Zambia	Mali	Haiti	Kenya	Nepal	Philippines	Iraq
11	Malawi	Togo	Kenya	Brazil	India	Nepal	Nepal
12	Congo	Tanzania	Mali	Cameroon	Ghana	North Korea	Morocco
13	Togo	Ghana	Laos	Nicaragua	Togo	Liberia	Syria
14	Madagascar	Gambia	Benin	Chad	Sierra Leone	Guinea	Egypt
15	Guinea	Lesotho	Timor-Leste	Malawi	Myanmar	Guyana	Iran

The BPI should not be used as a one-stop shop for making decisions on biofortification investment because it has several limitations. The main limitation is that the data are at a national level, i.e., at the highest level of aggregation for each country, meaning the BPI may overlook important within-country information. Another key limitation is the potential biases that may arise as a result of the aggregated consumption figures. It is likely that the national-level consumption figures are downward biased for rural households that are more likely to consume more staple crops than their urban counterparts. By the same token, it is also possible that consumption figures are upward biased because the target populations (especially children aged 6–59 months) consume less than the average person. Moreover, the BPI does not take into account differences in costs of breeding and delivery of biofortification programs across countries.

Some of these shortcomings will be addressed in future research. For now, the BPI is a useful tool for highlighting those countries that may benefit from significant reductions in micronutrient deficiencies through biofortification of staple crops.

To access the full BPI working paper, see: http://www.harvestplus.org/sites/default/files/working%20paper%20_11_web.pdf

National and International Standards and Regulatory Issues for Biofortification

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Biofortification is a relatively new process for increasing micronutrient levels of crops through breeding. HarvestPlus is currently leading the effort to develop and introduce biofortified staple crops in eight countries while also working with diverse partners in more than 40 countries. Currently, hundreds of thousands of households are being reached with biofortified foods, and global reach is rapidly increasing. As the effectiveness of biofortification is being demonstrated through the progress of these programs, national governments are adopting biofortification into agriculture and nutrition agendas, and global policies are being developed. Internationally recognized definitions, standards, and guidelines are needed to help formulate written policies and regulatory texts referring to biofortification.

Issues with Lack of Standards and Regulation

Without global standards, governments seeking to incorporate biofortification into national programs face significant challenges in regulation. Variations in food labelling, safety regulation and assessment of nutritional quality, product and crop naming issues, and guidelines for production provide barriers that complicate adoption of biofortification. This situation inevitably raises questions around what the trade challenges might be.

Standard Setting Process and the Codex Alimentarius

In order to address the critical need for internationally accepted standards and guidelines, HarvestPlus, through its association with the International Food Policy Research Institute (IFPRI), joined the Codex Alimentarius Commission to begin the process of introducing biofortification as a topic needing consideration by the appropriate Codex Committees. Established by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 1963, the Codex Alimentarius Commission oversees development of international standards, guidelines, and codes of practice for food to inform global and national texts.

Progress has been made in deliberating standards, although several issues have arisen that need consensus. Biofortification, as a definitional issue, was first introduced by HarvestPlus in 2012 to the Codex Committee on Food Labelling (CCFL). It was then reviewed in 2013 through a discussion paper. CCFL referred biofortification to the Codex Committee on Nutrition and Foods of Special Dietary Use (CCNFSDU), which then requested establishing a consensual definition of biofortification. In 2013, Zimbabwe and South Africa, both countries with biofortification initiatives and supported by 31 country interventions, offered to co-lead the development of a revised discussion paper and project document to request development of a formal definition.

This complex process includes considerations of how biofortified crops are distinct from their counterparts, potential types of organisms involved, inclusion of other attributes in addition to micronutrients (such as protein levels), and biofortification's relationship with fortification.

As this discussion continues, the next step will be to develop a Discussion Paper and Project Document by the Governments of Zimbabwe and South Africa on the need for a definition, to be presented at the next session of the CCNFSDU.

Highlights

- HarvestPlus/IFPRI gained “Member with Observer Status” in the Codex Alimentarius Commission.
- The CCFL has requested the development of a formal definition for biofortification by CCNFSDU.

Challenges

- Establishing an internationally agreed definition of biofortification.
- Having national governments reference an international standard on biofortification once it is developed.
- Establishing how Competent Authorities attest to the product.
- Establishing whether claims can be made once there is a definition.

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Consumer Acceptance of Biofortified Foods

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The success of biofortified staple crops depends on whether they are accepted and consumed by target populations. Several studies were undertaken in the past decade to understand consumers' acceptance of foods made with such staple crops. Consumer acceptance studies yield important information for breeding, product development, and demand creation activities. They specifically aim to answer two questions:

1. **Do consumers accept biofortified foods?** To gauge consumer acceptance of food made with biofortified varieties of staple crops, the following methods are used: (1) sensory evaluation (organoleptic) and hedonic tests, which measure consumer rating of various sensory attributes (e.g., taste, aroma, appearance, texture, cooking quality, etc.) of food made with biofortified varieties of crops compared to food made with conventional varieties; and (2) incentive-compatible economic experiments to elicit consumer willingness to pay (WTP) for biofortified varieties vis-à-vis conventional ones.

2. **What are the levers that influence consumers' acceptance of biofortified foods?** The impact of the following on consumers' acceptance of biofortified foods are tested: (1) nutrition information and the media channels used to convey such information; (2) length and content of nutrition information; (3) different branding options; (4) nature (national or international) of the branding/certification agency that is endorsing the biofortified staple crop; and (4) nature of the agency that is delivering planting materials for the biofortified staple crop.

Key results of these studies are detailed below:

Vitamin A Orange Sweet Potato (OSP)

- Sensory evaluation studies conducted in Uganda, Tanzania, Mozambique, and South Africa revealed that consumers liked the sensory attributes of OSP, as well as those of various products (e.g., bread, chips, and doughnuts) made with OSP. Several studies highlighted consumer preference for high dry matter content, and some found a negative relationship between provitamin A content and dry matter content.

- WTP and sensory evaluation studies conducted in rural areas of Uganda revealed that in the absence of nutrition information, consumer WTP for OSP was similar to their WTP for white-fleshed sweet potato. When nutrition information on the benefits of OSP was provided, however, consumers were willing to pay a substantial price premium for orange varieties relative to white ones. Another study conducted in Mozambique found that consumers were willing to pay a price premium for OSP and that their WTP was influenced by information on nutritional benefits. These studies highlight the importance of information campaigns in driving demand for OSP (1).

Vitamin A Orange Maize (OM)

- A consumer acceptance study was conducted in rural areas of Zambia (2). The study revealed that even in the absence of nutrition information, consumers are willing to pay a price premium for *nshima* made with OM, compared to *nshima* made with white and yellow maize varieties. Nutrition information, however, translated into higher WTP for OM.

- In the same study, two sources of media were tested to convey the nutrition message (simulated radio messaging and community leaders). Consumers who received the information through radio and those who received it from community leaders showed similar WTP values, implying that radio messaging, which is significantly less costly than face-to-face message delivery, can be used to convey the nutrition information.

- Another study conducted in rural Ghana found that consumer WTP for *kenkey* made with OM is less than that for either white or yellow OM, although nutrition information reverses this ranking (3). An information campaign will be key to driving consumer acceptance of OM in Ghana.

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Vitamin A Yellow Cassava (YC)

- A consumer acceptance study was conducted in Imo and Oyo states of Nigeria (4). In this study, *gari* made with two YC varieties was tested against local *gari*. One of the YC varieties was deeper in color than the other. The local *gari* tested was white in Oyo but yellow (mixed with red palm oil) in Imo, in accordance with regional preferences.
- Both hedonic tests of sensory attributes and WTP results revealed that in the absence of nutrition information in Imo, local *gari* was preferred to the *gari* made with either YC variety. Once consumers were told about the nutritional benefits of YC varieties, however, *gari* made with the deeper-colored YC was preferred. Nutrition campaigns are very important in this state.
- Both hedonic tests of sensory attributes and WTP results revealed that consumers in Oyo preferred the *gari* made with light YC even in the absence of nutrition information. Once consumers received the information about the nutritional benefits of YC varieties, light-colored YC remained the most popular variety, but *gari* made with deeper-colored YC was preferred over the local variety. In Oyo, the light-colored YC could become a popular variety even without nutrition campaigns.
- In this study, the nature of the delivery authority was also tested. Some of the consumers were told that the YC varieties were delivered by international authorities, and some were told that the federal government delivered these varieties. Imo consumers were indifferent to the authority delivering the biofortified planting material, whereas Oyo consumers preferred delivery by the international authority.

Iron Pearl Millet (IPM)

- A consumer acceptance study was conducted in rural Maharashtra, India, where *bakhri* made with IPM and market-purchased pearl millet were evaluated (5). Both organoleptic tests and WTP results reveal that even in the absence of information about the nutritional benefits of IPM, consumers liked the sensory attributes of the grain and *bakhri* of the IPM variety as much as (if not more than) those of the conventional variety.
- In this study, the impact on demand for IPM of state versus international brands and certification authorities was tested; consumers were found to be indifferent.

Iron Beans (IB)

- A consumer acceptance study conducted in rural Guatemala revealed that even in the absence of information about nutritional value, consumers preferred the sensory attributes of the IB variety to the local one. No evidence was found on the impact of information on WTP.
- Consumer acceptance studies were also conducted in rural Rwanda (6). Even in the absence of nutrition information, consumers in Western Province liked the sensory attributes of one of the IB varieties tested more than the local or other IB variety. Information on the nutritional benefits of IB varieties did not have a clear impact on consumers' preference for IB varieties. Likewise, length of the message (short versus long) and endorsement by a district leader did not have significant effects on consumer acceptance. In Northern Province, consumers were willing to pay more for one of the IB varieties compared to the local one even in the absence of nutrition information. However, the presence of information did not increase consumer WTP for the other IB variety tested.
- In urban wholesale and retail markets, consumers preferred one of the IB varieties more than the local and other IB varieties tested. With information on nutritional benefits of IB varieties, however, consumers preferred both IB varieties to the local one. Different messages tested (positive vs negative messages about the effects of having sufficient iron in diets) had a similar impact on consumer acceptance.
- Consumers in the urban wholesale market had similar preferences to those in the rural areas of Western province. This represents an opportunity for creating demand-pull from the urban areas since rural producers are more likely to produce what they prefer to consume and are thus more likely to produce the IB variety also preferred by urban consumers.

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2. Chowdhury, S; et al. 2011. Are consumers in developing countries willing to pay more for micronutrient-dense biofortified foods? Evidence from a field experiment in Uganda. American Journal of Agricultural Economics, 93(1): 83–97.
3. Banerji, A; et al. 2013. Using Elicitation Mechanisms to Estimate the Demand for Nutritious Maize: Evidence from Experiments in Rural Ghana. HarvestPlus Working Paper 10. Washington, DC: HarvestPlus.
4. Oparinde, A; et al. Forthcoming. Information and consumer willingness to pay for biofortified yellow cassava: Evidence from Experimental Auctions in Nigeria. HarvestPlus Working Paper. Washington, DC: HarvestPlus.
5. Banerji, A; et al. 2013. Information, branding, certification, and consumer willingness to pay for high-iron pearl millet: Evidence from experimental auctions in Maharashtra, India. HarvestPlus Project Report. Washington, DC: HarvestPlus.
6. Oparinde, A; et al. Forthcoming. Consumer Acceptance of High Iron Beans in Rural and Urban Areas of Rwanda: The Case of Kigali and Western Provinces. HarvestPlus Project Report. Washington, DC: HarvestPlus.

Cost-effectiveness of Biofortification

Ekin Birol, Dorene Asare-Marfo, Jack Fiedler, Barbara Ha, Keith Lividini, Mourad Moursi, Manfred Zeller (IFPRI – HarvestPlus); J.V. Meenakshi (Delhi School of Economics); and Alexander J. Stein (IFPRI)

Cost-benefit analysis (CBA) is often used to calculate and compare benefits and costs of a project, decision, government policy, or intervention (hereafter, “intervention"). CBA has two purposes: to determine if the intervention is a sound investment, and to provide a basis for comparing interventions. CBA involves comparing the total expected present cost of each intervention against the total expected present benefits to see whether the benefits outweigh the costs and by how much. For CBA, costs as well as benefits have to be expressed in monetary values. Cost-effectiveness analysis (CEA), on the other hand, compares the relative costs and outcomes (benefits) of two or more interventions. The intervention that achieves a certain (non-monetary) outcome at the least cost is usually preferred. In health literature, Disability-Adjusted Life Years (DALY) is often used to measure the outcomes of health interventions. The DALY is a measure of the overall disease burden of a particular condition (such as micronutrient deficiencies), expressed as the number of years lost due to ill-health, disability, or early death, according to the severity of the adverse health outcomes it entails.

With the metric “costs per DALY saved,” the costs of biofortification can be compared with other interventions, such as fortification. The World Health Organization’s CHOICE (Choosing Interventions that are Cost Effective) Working Group has suggested that a health intervention should be considered “very cost-effective” if its cost per DALY saved is less than national per capita income, and “cost-effective” if it is between 1 to 3 times per capita income.

Several CEAs have been conducted to gauge the feasibility and desirability of biofortification interventions (i) across micronutrient-country-crop combinations and (ii) compared to other micronutrient interventions within a country (1). The majority of these CEAs are ex ante (before the event) and are based on assumptions and projections of the future costs and coverage (adoption and consumption rates) of biofortification. These CEAs have long time horizons (about 30 years), as it takes time for the suitable biofortified varieties to become available, to be adopted and consumed on a large scale, and for health benefits to surface among the consuming population.

A recent study by Fiedler and Lividini estimates the ex ante effects of vitamin A orange maize, as well as five other interventions, such as fortified oil, sugar, and wheat flour in Zambia (2). It assesses the cost-effectiveness, total costs, and total number of DALYs saved from single interventions and from combinations of different interventions. The cost per DALY saved through vitamin A orange maize was estimated at US$24. Moreover, several of the intervention combinations cost less than US$50 per DALY saved. Perhaps the most important finding is that a combination of biofortification with some form of fortification can result in lower costs per DALY saved while increasing total numbers of DALY saved within a given budget. Biofortification is found to be especially cost-effective for maize-producing farmers whose families consume maize from their own production. Fiedler and Lividini also focus on zinc rice and zinc fortification of wheat flour in Bangladesh (2). Preliminary results reveal that the cost per DALY saved through zinc rice is less than one-third of the cost per DALY saved through fortification of wheat with zinc, suggesting that biofortification of rice is the most cost-effective, long-run strategy to sustainably reduce zinc deficiency in Bangladesh.

The preliminary results from recent ex ante CEAs on zinc and vitamin A crops are reported in the table below. For all crop-country combinations, biofortification can be rated as very cost-effective, as costs are significantly below per capita income, which ranges from US$365 in the Democratic Republic of Congo (DRC) to US$3,843 in India. When compared to fortification, biofortification is found to be more cost-effective for all crop-country-micronutrient combinations. Moreover, for all cases except one, biofortification is more cost-effective than supplementation.

Ex post (after the event) CEA seeks to measure cost-effectiveness of an already implemented intervention and uses actual data on the costs and coverage (adoption and consumption) rates of the intervention. The CEA of the Reaching End Users in Uganda with orange sweet potato (OSP) project (2006–2009) found this intervention’s cost per DALY saved to be in the range of $US15–24 (6). Further evidence on ex post cost-effectiveness of biofortified crops will be built in the coming years.

The CEA conducted thus far reveals biofortification to be a highly cost-effective strategy for reducing micronutrient deficiencies. It is, however, important to note that the cost per DALY saved cannot be used as the sole criterion for determining the most appropriate micronutrient intervention. This is because it does not consider the total number of DALYs that can be saved under a given budget. A given intervention may be inexpensive but may also have small coverage and save only a few DALYs. Likewise, an intervention that is more cost-effective overall may be relatively less cost-effective in certain scenarios (target groups, time horizons, available infrastructure, etc.). Therefore, a combination of biofortification, supplementation, and fortification may be

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best for achieving cost-effective and large scale impact. The cost advantage of biofortification comes from the economies of scale (once a new crop has been developed, its benefits can be spread relatively cheaply over time and space) and lies in its ability to reach a high number of rural (farming) households who produce and consume large amounts of staple food crops and who suffer from micronutrient deficiencies.

Table 1. Cost-effectiveness of Biofortification

Country	Micronutrient	Biofortification Cost per DALY Saved*	Fortification Cost per DALY Saved**	Supplementation Cost per DALY Saved***
Bangladesh	Zinc	Rice: $11-32	Wheat: $19	$7
India	Zinc	Rice: $0.6-2	Wheat: $16	$7
Pakistan	Zinc	Wheat: $3-18	Wheat: $27	$58
India	Zinc	Wheat: $1-4	Wheat: $16	$7
DRC	Vitamin A	Cassava: $0.4-1	Sugar: $37	$52
Nigeria	Vitamin A	Cassava: $0.3-0.5	Sugar: $50	$52
Ethiopia	Vitamin A	Maize: $2-6	Oil: $43	$52
Zambia	Vitamin A	Maize: $9-30	Wheat: $12	$52
Uganda	Vitamin A	Sweet potato: $4-7	Sugar: $56	$52

* Preliminary results by Birol et al. (2014) (3); ** Fiedler and Macdonald (2009) (5); ***WHO CHOICES (50% coverage) (5).

Biofortification interventions exhibit relatively high up-front costs in the first six to 10 years. These costs depend on many factors, such as the type of crop and micronutrient, size of the target country, research infrastructure, and seed sector. As HarvestPlus and its partners make further progress in breeding and identifying cost-effective models for delivery, the costs to cover new regions and countries will decrease. In these new countries, however, there will still be the costs of adaptation and maintenance breeding and establishing seed multiplication and delivery channels. These costs could, to some extent, be alleviated through cross-country learning and spillover. The early phases of biofortification interventions generate public goods (e.g., knowledge about biofortified crops, breeding lines with high micronutrient content), which will need to be financed by governments and donors. In later phases, nutritional value becomes a standard target (i.e., mainstreamed) in public breeding programs, and biofortified germplasm is shared with the private sector, which can integrate it in its breeding programs. In the long run, biofortification can be self-sustaining.

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2. Fiedler, J; Lividini K. 2014. *Zambia and Bangladesh micronutrient portfolio analyses*. Washington, DC: HarvestPlus.
3. Birol, E; et al. 2014. *Cost-effectiveness of biofortification revisited*. Washington, DC: HarvestPlus.
4. Fiedler, J; Macdonald, B. 2009. A strategic approach to the unfinished fortification agenda: Feasibility, costs, and cost-effectiveness analysis of fortification programs in 48 countries. *Food and Nutrition Bulletin* 30(40):283–416.
5. WHO CHOICES. http://www.who.int/choice/en/
6. HarvestPlus. 2010. *Disseminating orange-fleshed sweet potato: Findings from a HarvestPlus project in Mozambique and Uganda*. Washington, DC: HarvestPlus.

Bridging Agriculture and Nutrition: Challenges in Communication

Gary R. Gleason, PhD (Independent Consultant)

Context

By the 1970s, large-scale agriculture improvement efforts included strategic communication components. Outputs ranged from carefully designed media products to training for extension officers on group and interpersonal communication. Human nutrition tended to be better linked to agriculture in countries where the extension approach had roots in the British system that included “home economics” as a specialty in agricultural extension.[i] Communication support for many agricultural innovations routinely included instructions and promotion on the use of an innovative product or processes to improve family nutrition. Communication strategies typically included audience research and segmentation, message formation, channel mix, and feedback as outlined in “communication of innovation” models (1). In the 1990s and 2000s, agriculture sector extension staff became smaller, and communication strategies shifted toward informational materials on specific innovations and crops, as well as wider use of “two-step early adopter” communication models such as Farm Field Schools and “lead farmer” systems.

Independent of the agriculture sector, nutrition communication activities matured in the 1980s, carried by the “Child Survival Revolution.” In the 1990s, emphasis shifted toward a balance of social and psychological models, with channels and messages focused on improving feeding practices and how food was used. “Social marketing” models promoted nutrition-related products, such as iron folate supplements and fortified staples, across large populations, and “positive deviance” became widely used at the community level to promote and demonstrate improved nutrition-related practices.

Until recently, most nutrition communication strategies stayed on their own side of the agriculture-nutrition bridge. Sophisticated nutrition communication work focused on establishing and reinforcing food as a fundamental human right, promoting food fortification (including iodization of salt), battling industries that sold and promoted breast milk substitutes, and improving infant and young child feeding—particularly breastfeeding. Agriculture communication strategies and products also stayed primarily on their own side of the bridge and targeted improving crops for cash and/or consumption, improving group communication channels through Farmer Field Schools, promoting subsidized inputs programs, and protecting land through improved cropping techniques.

Current Status

In 2014, communication challenges are no longer isolated in either “nutrition” or “agriculture.” Agriculture in developing countries has moved beyond cash crops and subsistence farming, and nutrition is no longer focused almost exclusively on acute malnutrition, hidden hunger, and Infant and Young Child Feeding (IYCF).

While eliminating extreme poverty by 2030 is a central post-2015 development agenda issue, the International Food Policy Research Institute (IFPRI) calls for equal importance to be given to eliminating hunger and undernutrition, targeting the 12 percent of humanity who suffers from hunger on any given day and the more than two billion who are affected by micronutrient deficiencies. IFPRI says this can be achieved by 2025. The related communication challenge, then, is not only to fully link agriculture with nutrition, but to also build strategies that effectively balance support for promoting both social and individual behavioral change.

In the context of enhanced national food and nutrition priorities, the concept most often used to bridge the two is food security. While food security is necessary for nutrition and health, the concept is complicated and often misused. Too often agricultural policymakers will accept the linkage of agriculture with nutrition while limiting their sector’s contribution to the development, production, and increased access of households to nutritious foods. Those working on the nutrition side, most often from the health sector, tend to be more oriented toward feeding practices and prevention of infection than on continuous availability, accessibility, preparation, and use of nutritious foods.

In recent years, communication strategies have followed areas where food and nutrition have gained substantial national and international support. These include policy development, the work of food processors in fortification, the development of more nutritious crop varieties, and the promotion of multi-sector programs. Strategic communication support in these areas has been well planned, multi-channeled, evidence based, and reasonably effective.

At the policy level, Kenya’s Food and Nutrition Policy, Rwanda’s updated Food and Nutrition Policy, and Zambia’s National Food and Nutrition Strategic Plan each have clear, high-priority linkages between food and nutrition. These linkages include fortification and food processing at the national level, decentralized district plans to reduce malnutrition, and food- and nutrition-oriented social assistance targeting the most vulnerable populations.

Complementing many such food and nutrition policy linkages is the recent strategic emphasis on the first 1,000 Days of a child's life, from the start of a woman's pregnancy until her child's second birthday. New evidence and communication strategies show impacts of stunting on fetal and child development, adult health, and national economic productivity. The overarching goal of the 1,000 Days initiative, to prevent chronic malnutrition and support the long-term health and development of children aged under two, serves as a powerful tool for communication strategies to link agriculture and nutrition and support change at all levels.

Countries are also developing multi-sector strategies and communication support that link household food production with the nutrition requirements and healthy eating practices for pregnant women and young children. Communication support for 1,000 Days programs requires multiple partners and effective messages that link diverse intervention packages. These may include backyard nutrition gardens, biofortified crops, small livestock, fortified staples, micronutrient powders for home fortification, focused antenatal care, vitamin and mineral supplementation, maternal, infant, and young child feeding, sanitation and hygiene, infection prevention, and several others.

Multi-sector interventions and communication support are essential because no single intervention or set of interventions from any one sector can substantially lower the prevalence of stunting. The required linkages include, at minimum, agriculture, health, and social assistance, as well as communities and other partners. Success will require both strengthened services and interrelated, mutually reinforcing communication strategies, activities, and products that facilitate adoption of improved practices at both the community and household levels.

Challenges

Coordination across sectors may be unfamiliar. For example, one national Food and Nutrition Strategy is explicitly owned by the ministries responsible for health, agriculture, and social assistance. Two of these ministries have dedicated, well-equipped, and professionally staffed communication support units. Despite an ongoing national 1,000 Days communication campaign, a major 1,000 Days multi-sector component in current National Food and Nutrition Strategic Plan, and a new Nutrition Action Plan from the agriculture sector, the leaders and staff of these units have not met for joint planning and discussions.

Strategies for Strengthening Communication Support across the Agriculture-Nutrition Bridge

- Recognize and address communication gaps by explicitly raising nutrition considerations for each link in the chain from national food economies to households and individuals.
- Recognize that the human right to food and nutrition requires elimination of the measurable risks and potential lifelong damage to a child's growth, health, cognitive development, and productivity that result from inadequate and unbalanced nutrition during the first 1,000 Days.
- Recognize that ensuring adequate nutrition often involves at least three supporting sectors of services and related information including: 1) agriculture, 2) health, and 3) social assistance.
- Select and link essential services and information from across sectors in a manner that encourages and facilitates learning, trial, and adoption of a sequence of practices and behaviors that positively affect health and nutrition. This step will need to be continued or carried out concurrently until a fully adequate set of practices and essential behaviors is identified (there will be additional alternatives).
- Evaluate, and if necessary improve, the knowledge and services needed to support adoption of each effective practice and essential behaviors.
- Develop and implement effective, science-based communication strategies to promote these steps including, as needed, components aimed at:
    - Policy advocacy and resource generation,
    - Organizational motivation and alliance building, and
    - Social and individual behavioral change.

1. Ascroft, J; Gleason, G. 1979. *Establishing the information support unit, Ministry of Agriculture*. Report of Project Information Support Unit of the Ministry of Agriculture for Development Support Communication Department. Rome: United Nations Food and Agricultural Organization (FAO)

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[i] "Home Economics" was a component of the British colonial system of agricultural extension. This component or specialist was not present in the French colonial system, nor is there an equivalent in many former Francophone countries.

PROGRESS BRIEF #27

NUTRITION & IMPACT

Recent Rising Food Prices Have Resulted in Severe Declines in Mineral and Vitamin Intakes of the Poor

Howarth Bouis (IFPRI-HarvestPlus)

In terms of energy intakes, the diets of the poor in developing countries are dominated by consumption of food staples, as shown in the example of Bangladesh in Figure 1. However, minerals and vitamins are concentrated in more expensive non-staple plant and animal foods. Minerals and vitamins in animal foods are highly bioavailable.

Expenditures on non-staple foods by poor consumers comprise 40–60 percent of total expenditures for food. Because of poor dietary quality (low intakes of non-staple plant and animal foods), intakes of vitamins and minerals are too low, resulting in high prevalence rates of micronutrient deficiencies.

Because of the Green Revolution, which resulted in rapid increases in cereal yields that grew faster than the population (see Figure 2), cereal prices fell markedly from the early 1970s to the mid-1990s but have risen again as productivity increases have slowed. Non-staple food prices, on the other hand, have been rising steadily throughout this period (Figures 3 and 4).

The poor in developing countries cope with rising food prices in two primary ways: (1) by reducing the amount of expensive meats, dairy, fruit, vegetables, and pulses (non-staple foods) consumed, resulting in large declines in mineral and vitamin intakes; and, (2) by reducing expenditures on non-food items, such as education, housing, and medical care. This is shown in Figure 5 for Bangladesh, based on the data shown in Figure 1.

Demand for food staples (e.g., rice, wheat, maize, depending on the geographical region and culture) is highly inelastic. That is, in the case of Bangladesh, the poor continue eating about the same amount of rice to keep from going hungry. They must spend more on rice due to rising prices and so must spend less on even more expensive, non-staple foods.

For Bangladesh, a 50 percent increase in all food prices across the board (holding income constant) will result in a 30 percent decline in iron intakes. This, in turn, will result in an increase in the prevalence rate of iron deficiency among women and children. Modest decreases in present intakes of minerals and vitamins will drive these prevalence rates significantly higher, with severe consequences on the nutritional status of the poor and on public health (Figure 6).

Dietary sources of minerals and vitamins have become more expensive over time. By putting more minerals and vitamins in staple foods ***at no extra cost to consumers***, biofortification helps to mitigate the harmful effects of rising food prices on dietary quality. A much larger challenge is to increase the productivity of a long list of non-staple plant foods and animal products.

Figure 1. Energy Source and Food Budget, Bangladesh

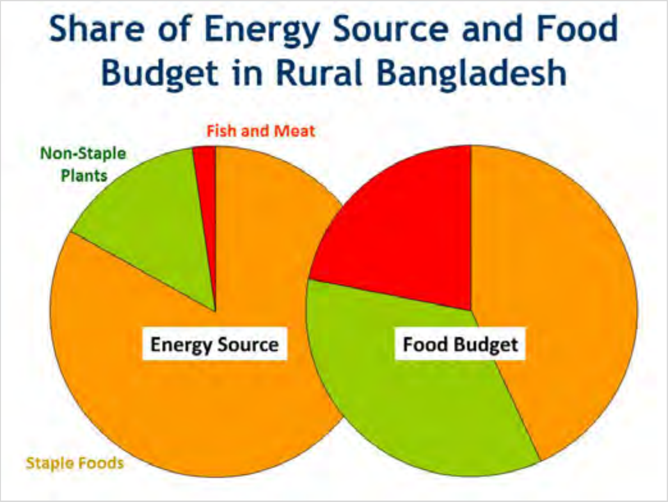

Figure 2. Changes in Cereal and Pulse Production

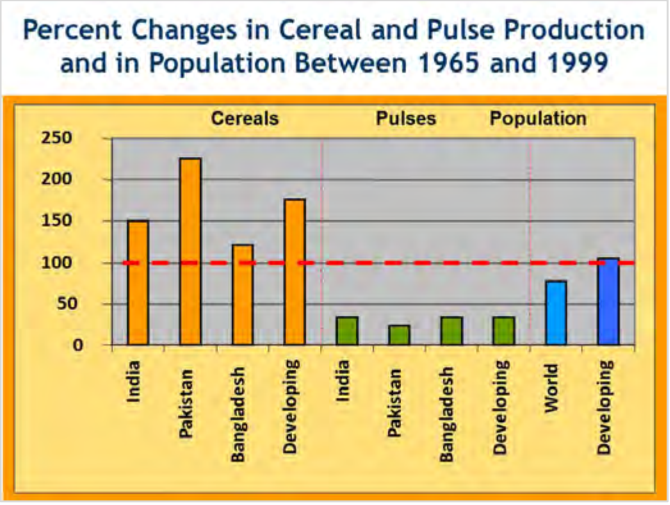

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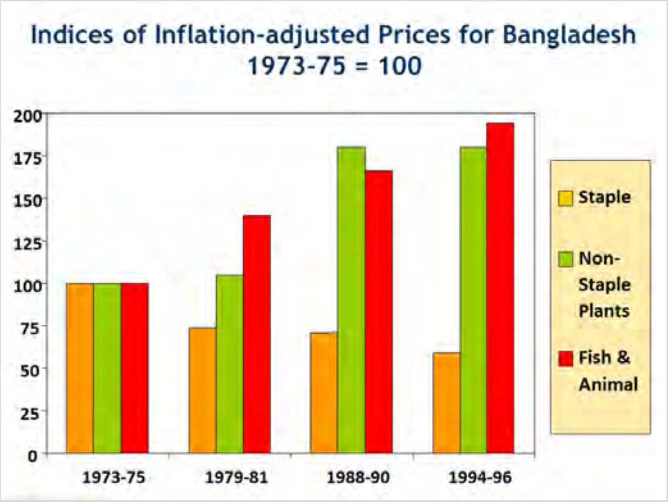
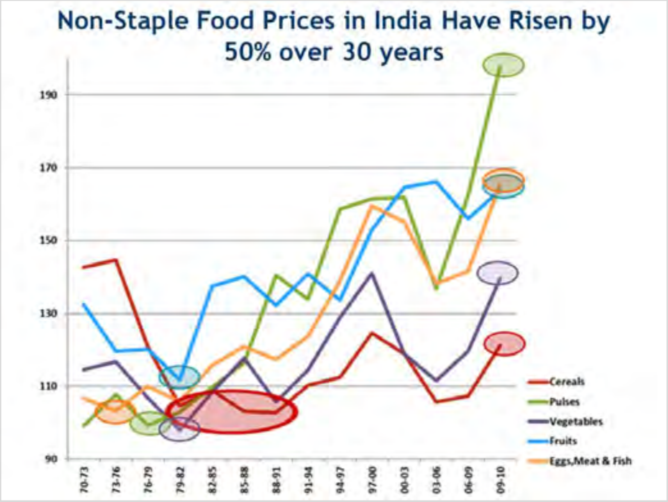
Figure 3. Inflation-adjusted Prices, Bangladesh



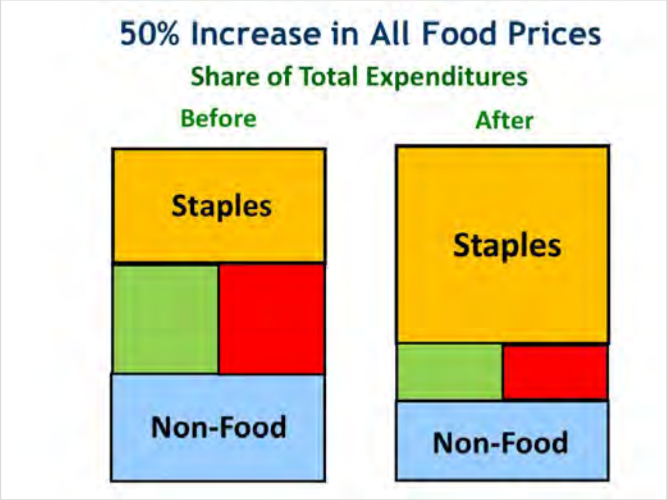
Indices of Inflation-adjusted Prices for Bangladesh 1973-75 = 100

Figure 4. Rise of Non-Staple Food Prices in India



Non-Staple Food Prices in India Have Risen by 50% over 30 years

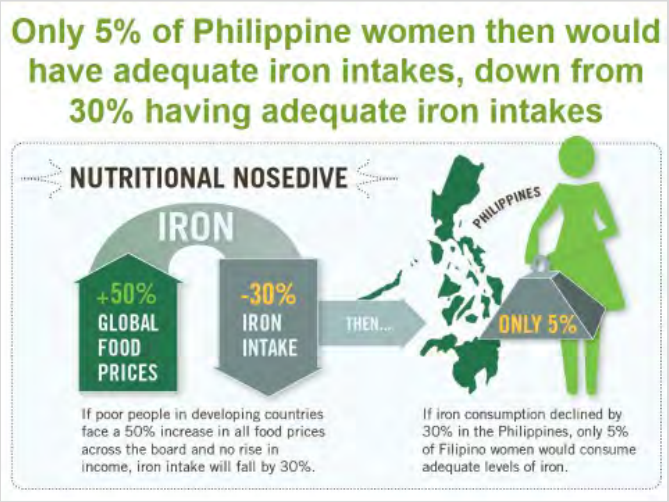
Figure 5. Increase of Food Prices



50% Increase in All Food Prices

Share of Total Expenditures

Figure 6. Iron Intake and Deficiency



Only 5% of Philippine women then would have adequate iron intakes, down from 30% having adequate iron intakes

If poor people in developing countries face a 50% increase in all food prices across the board and no rise in income, iron intake will fall by 30%.

If iron consumption declined by 30% in the Philippines, only 5% of Filipino women would consume adequate levels of iron.

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Breeding for Improved Micronutrient Bioavailability & Gut Health

Ross M. Welch (Cornell University)

Edible portions of staple food crops contain certain constituents (antinutrients) that can inhibit the absorption and/or utilization (i.e., bioavailability) of divalent and polyvalent trace element cations (e.g., Fe^{3+}, Zn^{2+} and Cu^{2+}) from these foods and from other dietary components in a meal. These foods can also contain compounds ("promoters") that enhance or promote the bioavailability of essential trace elements even when antinutrients are present in a meal. Phytic acid is the most studied antinutrient. It can bind to these cations along with other dietary components because of its large negative charge, making the cations insoluble and unavailable for absorption by mucosal cells in the gut. Levels of these antinutrients and promoters in plant foods can differ depending on both genetic and environmental factors. Current plant molecular, biological, and genetic modifications, combined with plant breeding approaches, now make it possible to reduce or eliminate antinutrients from staple plant foods, or to significantly increase the levels of promoter substances in these foods.

Some promoter compounds are normal plant metabolites and fewer genes control their levels in plants compared to the numerous genes required to regulate (via homeostasis mechanisms) the absorption, translocation and deposition of iron, zinc, and other essential trace minerals in edible portions of staple food crops. Only small changes in their accumulation in edible plant products may be required to have significant effects on the bioavailability of micronutrients. Thus, breeding for increased levels of these promoters should be relatively simple compared to breeding for higher concentrations of essential trace minerals in staple seeds and grains, which involve numerous genes and their interactions with the environment. Below are discussions of the most promising promoter compounds found in plants.

Prebiotics

Staple food crops can contain prebiotics (i.e., food substances that simulate the growth of beneficial microbiota (probiotics) in the human gut). The most studied of these prebiotics are the non-digestible carbohydrates such as inulin (a fructooligosaccharide). Prebiotics have been shown to have positive effects on enhancing the bioavailability of some mineral nutrients (e.g., iron, zinc, calcium and magnesium) in plant foods. The effects of human gut microbiota and their effects on human nutrition and health are just beginning to be recognized but not understood with any clarity. Undoubtedly, the effect of intestinal microbiota on our ability to utilize food, nutrients, and phytochemicals is immense.

The human intestine alone contains more microbiota than the eukaryotic cells of the entire body (i.e., at least 10 trillion intestinal microbial cells compared to about one trillion body cells). Metabolic activity of gut microbiota is equivalent to that of the body's vital organs. Microbial mass can account for 60 percent of the dry weight of feces. Studies have shown that microbial interactions with human cells are essential to normal mammalian physiology including metabolic activity and immune homeostasis. Gut microbiota provide energy from undigested food substrates, train the immune system, prevent growth of pathogens, transform certain nutrients and beneficial phytochemicals into utilizable substrates, synthesize certain vitamins, defend against certain diseases, stimulate cell growth, prevent some allergies, improve mineral absorption (e.g., iron and calcium) and produce anti-inflammatory effects. Most importantly, beneficial microbiota improves gut health in general, reduces gut inflammation, and improves the ability of people to utilize all nutrients in a meal more efficiently.

Changing gut microbiota populations to increased numbers of probiotic bacteria through dietary means by providing prebiotics in staple food crops appear to have enhancing effects on iron, zinc and other essential trace element bioavailability. Providing enhanced levels of prebiotics may overcome the negative effects of antinutrients on essential trace metal bioavailability because many bacteria in the gut can degrade antinutrients, such as phytate and polyphenols, releasing their bound metals and allowing absorption by enterocytes lining the intestine. Probiotic systemic effects on inducing the genes controlling the absorption of iron and other essential trace elements from the intestine can enhance the bioavailability of these essential trace elements to humans. Of equal and possibly more importance is the role of prebiotics in improving gut health and the intestine's ability to absorb and utilize numerous nutrients, regulate the immune system, and protect against invasion by pathogenic organisms. Thus, increasing the levels of prebiotics in staple food crops could be an extremely important strategy to enhance the nutrition and health of malnourished people worldwide.

Inulin and other non-digestible carbohydrates are common constituents of cereal grains and legume seeds. The biosynthetic pathways and genes associated with their accumulation in food crops have been explored. It is now possible to manipulate genes in staple food crops to accumulate significant amounts of some prebiotics (e.g., inulin) in their edible tissues. More research is needed to determine what types and amounts of prebiotics are required to enhance the bioavailability of iron, zinc, and potentially other micronutrients in staple food crops. This type of research should be expanded. Breeders should be informed and begin to enhance prebiotics in target crops to levels that would have health impacts.

Nicotianamine

Nicotianamine is a non-protein amino acid biosynthesized by all higher plants. It is the only known low molecular weight compound in plants that forms stable complexes with ferrous iron (Fe^{2+}). It is required for the cellular, intercellular, and long distance transport of iron throughout the plant. Several studies have shown that increasing nicotianamine levels in plants through molecular transformations results in greatly increased iron concentrations in seeds. Further, the iron accumulated in the seed as the iron(II)-nicotianamine complex appears to be highly bioavailable. Therefore, nicotianamine appears to be an ideal promoter of iron bioavailability and efforts should be started to find ways to increase its levels in seeds and grains of target crops in the HarvestPlus biofortification program.

Phytoferritin

All aerobic organisms store excess iron in a 450 kDa protein of 24 subunits – ferritin (i.e., phytoferritin in plants). This protein stores up to 4,500 ferric-iron atoms in its core. It accumulates in various cell plastids including chloroplasts. Excess plant iron is stored in phytoferritin. The iron stored in ferritin is bioavailable and does not interact with antinutrients present in a meal. Ferritin-iron is transported into gut mucosal cells *in toto* by binding to gut membrane sites and absorbed via endocytosis. Phytoferritin is an ideal promoter for inclusion in staple food crop biofortification programs.

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PROGRESS BRIEF #29

CROP DELIVERY

Delivery of Orange Sweet Potato (OSP) in Uganda

Anna-Marie Ball (IFPRI-HarvestPlus) & Jan Low (CIP)

Table 1. Staple Food Production & Consumption in Uganda

Staple Food	**Daily Per Capita Consumption** *(all age groups, grams/day)*[1]	**Total Annual Production** *(thousand metric tons)*[2]
Cassava	264 g/d	4,924
Sweet Potato	199 g/d	2,650
Maize	61 g/d	2,734
	Daily Per Capita Consumption *(grams/day)*[3]	**Provitamin A Density and Intakes**
Sweet Potato	Children (4–6 years): 100 g/d Women: 200 g/d	White Sweet Potato: 2 parts per million (ppm) Provitamin A Target Increment: +30 ppm Biofortified Sweet Potato Target Density: 32 ppm
		At the target level, biofortified sweet potato provides about 70–100% of the Estimated Average Requirement (EAR).

[1]FAO Stat 2009; [2]FAO Stat 2012 [3]HarvestPlus Surveys

Table 2. Current Vitamin A Status

Prevalence of Vitamin A Deficiency (2011 UDHS)	Children under five: 38%

Varietal Release

In addition to two vitamin A-rich local varieties (Ejumula and Kakamega), which were identified and released prior to the start of the HarvestPlus activities in the country, two OSP varieties with the full provitamin A target, NASPOT 9 O (VITA) and NASPOT 10 O (Kabode), were released in 2007. In 2013, two other OSP clones (SPKOO4/2006/1136 and NAS7/2006/292) were released as NASPOT 12 O and NASPOT 13 O, respectively. New OSP varieties are subject to both on-station and multi-location evaluation as part of the release process. Candidate varieties are also evaluated before release and bulking of vines in on-farm trials for palatability and acceptance tests, with farmers participating in the project. It is expected that there will be more OSP releases after 2014.

Table 3. Released OSP Varieties

Variety	Release Date	Mean Yield (tons/ha)	Dry Matter (%)	Fresh Weight (µg B-carotene (BC)/100g)	Fresh Weight (µg all-trans BC/g)	Dry Weight (µg all-trans BC/g)
Kakamega	2004	9.5	35.0	4,071	41	116.3
Ejumula	2004	6.0	34.6	9,062	91	261.9
NASPOT 9 O (VITA)	2007	8.5	30.3	7,460	75	246.2
NASPOT 10 O (Kabode)	2007	10.4	30.7	9,655	97	314.5
NASPOT 12 O	2013	12.2	32.7	7,230	72	221.1
NASPOT 13 O	2013	12.5	31.2	9,450	95	302.9

Strategic Factors Driving Delivery

The level of vine commercialization is variable across the target areas. Activities are underway to popularize the use of clean vines and encourage farmers and institutions to buy quality declared planting materials by demonstrating their performance, advertising multipliers who sell OSP varieties, and enabling the use of low-cost irrigation technologies to ensure vines are available when farmers need them.

Seed Commercialization

HarvestPlus initially focused on production and consumption at the household level. With increasing market presence, HarvestPlus has initiated market linkages and business support activities for OSP roots and vines. Training materials have been developed for

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extension workers and farmers, and HarvestPlus has facilitated linkages between farmer groups and traders. Given these additional activities, HarvestPlus is working to supply urban markets by expanding to more districts where sweet potato production is already high. Deliberate effort was made to work with farmers who are aware of the market dynamics for sweet potato; HarvestPlus supplied these farmers with OSP vines that could be integrated into their production systems. In the absence of a highly commercialized vine market in Uganda, HarvestPlus is nurturing a strong public-private partnership aimed at maintaining production and supply of clean OSP planting materials that are accessible to farmers. Vine commercialization channels include direct marketing, cooperatives, and small-scale vine dealers.

Marketing

Educating household decision makers on the recommended feeding practices for children under five and mothers, as well as the health benefits associated with consuming these crops, creates awareness and demand for OSP vines. Building product acceptance is further facilitated by outreach activities in the target communities and mass awareness campaigns, as well as demonstrations of the agronomic superiority of the nutrient-dense varieties compared to older varieties currently farmed. Promotional campaigns targeting urban consumers, traders, and retailers are conducted in markets and on the radio in tandem with production seasons.

Photo: HarvestPlus

Stakeholders

In supplying virus-free planting materials, HarvestPlus, in partnership with Makerere University and International Potato Center (CIP), has engaged with BioCrops Ltd., a private tissue culture laboratory, to clean and multiply tissue culture OSP vines, which are then sold to farmers and public sector or nongovernmental organization (NGO) vine producers. Implementation of the project is done through development partners, including: World Vision, Samaritan’s Purse, Caritas, Africa 2000 Network-Uganda, Volunteer Efforts for Development Concerns (VEDCO), Millennium Village Project–Ruhira, Community Enterprise Development Organization (CEDO), and Mbarara University of Science and Technology (Healthy Child Project) with additional support from Farm Radio International and TRAC FM.

Potential Impact

HarvestPlus and its partners have been disseminating OSP in Uganda since 2007, reaching more than 149,000 farming households for whom sweet potato is a staple food. The project has generated extensive evidence on the impact of growing and consuming OSP on vitamin A intake and status of women and children. The medium-term objective is to reduce micronutrient malnutrition and improve dietary intakes of vitamin A for 237,500 households in 20 districts of Uganda by 2018.

Cost

HarvestPlus will spend an estimated total of US $7.5 million for OSP delivery activities in the period 2013–2018.

Delivery Challenges and Recommendations

- Because sweet potato vines are bulky and highly perishable, it is best practice to minimize moving them over long distances. Therefore, individuals and farmer groups in target areas have been identified to handle vine multiplication.

- Government support is absolutely critical for sustainability both in terms of integrating biofortification into agricultural and social sector policies and programs, and creating regulations and standards that support private sector development and engagement. In addition to public support, the government must create a regulatory environment and policies that are conducive to private sector participation in developing sustainable vine and root markets in Uganda.

Delivery of Pearl Millet in India

Binu Cherian (CIAT-HarvestPlus)

Table 1. Staple Food Production & Consumption in India

Staple Food	Daily Per Capita Consumption *(all age groups, grams/day)*[1]	Total Annual Production *(thousand metric tons)*[2]
Rice	187	152,600
Wheat	158	94,880
Pearl Millet	17	10,330
Maize	14	21,060
Irish Potatoes	58	45,000
	Daily Per Capita Consumption *(grams/day)*[3]	**Iron Density**
Pearl Millet	Children (4–6 years): 150 g/day Women: 300 g/day	Conventional Pearl Millet: 47 parts per million (ppm) Iron Target increment: +30 ppm Biofortified Pearl Millet Target Density: 77 ppm
		At the target level, biofortified pearl millet provides about 100% of the Estimated Average Requirement (EAR).

[1]FAO Stat 2009; [2]FAO Stat 2012; [3]HarvestPlus Surveys

Table 2. Current Iron Status

Prevalence of Anemia (2005-06 NFHS)	Children (6–35 months): 70% Pregnant women 59% Non-pregnant women: 55%

Varietal Release

In 2012, HarvestPlus partner Nirmal Seeds commercialized ICTP-8203-Fe, an improved version of existing variety ICTP-8203, a regionally well-adopted crop with more than 90 percent of the iron target, in the state of Maharashtra. This improved version is on average 15 percent higher yielding (which will drive replacement of ICTP-8203 with ICTP-8302-Fe) and has 11 percent higher iron. ICTP-8203-Fe (Dhanshakti) has been officially released and notified in 2014. HarvestPlus will also collaborate with the publicly run Maharashtra Seeds Corporation Limited in seed production and distribution. In parallel, iron pearl millet hybrids are being developed for commercialization, starting in 2014, in partnership with seed companies Shakti Vardhak Hybrid Seeds and Nirmal Seeds. This initiative has a target of reaching at least 1.5 million farming households and 14 percent of the pearl millet market share by the end of 2018. Delivery channels will include both private and public sector seed companies.

Figure 1. Example of Seed Pack Design and Logo

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Strategic Factors Driving Delivery

In India, strong pearl millet breeding programs exist in both the public and private sectors. The commercial market is largely driven by hybrids (95 percent) with the private sector playing a key role. Hence, HarvestPlus engages both actors in mainstreaming iron pearl millet. Private sector seed companies have strong crop research and development programs, especially for crops that provide hybrid cultivar options.

Seed Commercialization: To ensure long-term sustainability, seed companies must engage in iron pearl millet breeding and establish their own high-iron product lines. Therefore, the HarvestPlus strategy engages seed companies in genotype-by-environment (GxE) testing of hybrids and inbred lines developed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and encourages companies to develop their own high-iron hybrids for commercialization. Private seed companies operate a two-tier distribution system, supplying seed directly to distributors who, in turn, sell to retailers. Generally, each distributor sells to 40–50 retailers, depending on location and crops/products. These retailers ultimately sell the seeds to farmers. Pearl millet seeds are sold commercially in 1.5 kilogram and 3 kilogram packs, in attractive primary packaging with mandatory labeling (according to India's Seed Act).

Marketing

Demand for iron pearl millet seed is created by farmer demonstrations, field days, and promotions at points of sales. Nirmal Seed's channel partners and personnel have been trained in nutrition messaging for iron pearl millet, a crucial component in the delivery process. Building product acceptance is further facilitated by the agronomic superiority of a recently released high-iron variety compared to an older version currently farmed. HarvestPlus initially focused on seed sales, but with increasing market presence HarvestPlus will collaborate with various actors in food and retail to create demand for iron pearl millet grains, flour, and value-added products. This includes testing specific promotional messages and product benefits, communication channels and their effectiveness, and the selection of the brand name and advertising.

Stakeholders

Partnerships are extremely important to the delivery efforts in India, and HarvestPlus works closely with ICRISAT, five State Agricultural Universities, and 15 seed companies – most notably Nirmal Seeds and Tempest India for crop delivery.

Government Support

The Indian government has endorsed the use of nutrient-rich crops and earmarked about US $40 million in the budget for 2013-14 to establish nutri-farms, where iron pearl millet, zinc rice and wheat, and protein-rich maize will be grown. Nutri-farms will promote, encourage, and develop commercial cultivation and strengthen the supply chain of nutrient-rich crops to reach vulnerable sections of the population.

Potential Impact

At the end of 2013, a cumulative 25,000 farming households in India had been reached with iron pearl millet. It is projected that ICTP-8203-Fe market share in India will reach the saturation stage by 2015, and iron pearl millet will enter the anchoring stage of delivery by 2018. In Rajasthan and Maharashtra, where 65 percent of the production is concentrated, the projected iron pearl millet market share by 2018 is 14 percent. It is estimated that 1.17 million farming households will have access to iron pearl millet by 2018.

Delivery Challenges and Recommendations

- Incorporating iron pearl millet grains in the existing institutional government programs, e.g., subsidized public food distribution and mid-day meal programs for school children
- Creating demand for increasing consumption of iron pearl millet grains on farm and market linkages for grains and value-added products
- Developing technologies to increase the shelf life of pearl millet flour

PROGRESS BRIEF #31

CROP DELIVERY

Delivery of Zinc Wheat in Pakistan

Qadir Baloch (CIAT-HarvestPlus)

Table 1. Staple Food Production & Consumption in Pakistan

Staple Food	**Daily Per Capita Consumption** *(all age groups, grams/day)*[1]	**Total Annual Production** *(thousand metric tons)*[2]
Wheat	302 g/d	23,473
Rice	70 g/d	9,400
Irish Potatoes	37 g/d	4,104
	Daily Per Capita Consumption *(grams/day)*[3]	**Zinc Density**
Wheat	Children 3–4 years: 72 g/d Women: 208 g/d	Conventional Wheat: 25 parts per million (ppm) Zinc Target Increment: +12 ppm Biofortified Wheat Target: 37 ppm
		At the target level, biofortified wheat provides 70-100% of the Estimated Average Requirement (EAR).

[1]FAO Stat 2009; [2]FAO Stat 2012; [3]HarvestPlus Surveys

Table 2. Current Zinc Status

Prevalence of zinc deficiency (NNS 2011)	Children under five: 37%

Varietal Release

In Pakistan, breeding of biofortified zinc wheat is at an advanced stage at the Pakistan Agricultural Research Council (PARC), Islamabad. Three high-zinc leads with +6–14 ppm zinc were submitted for official registration trials in 2012/13, with release expected in 2015. The first biofortified candidate variety, NR-421, is in the second year of testing in National Trials and is anticipated to be officially released in 2015 for general cultivation. Another lead, NR-419, is also high yielding, has more than 37 ppm zinc and 70 ppm iron, and is high in protein content as well. It is in the first year of testing in National Trials (irrigated). The third potential wheat line, NR-439, with similar agronomic characteristics as NR-419 is in the first year of testing in rainfed areas of the country.

Table 3. First-Wave Wheat Candidates for Release in 2014/15

Variety Name	**Grain Yield**		**Zinc Increase (ppm)**		**Agronomic Performance**[2]		
	t/ha[1]	**% over check**[1]	**Pakistan 2 sites**	**Asia 7 sites**	**Heading (days)**	**Maturity (days)**	**Height (cm)**
NR-419	4.5	110%	+9.1 ppm	+6.6 ppm	93/94	130/127	105/81
NR-420	3.4	84%	+7.3 ppm	+6.8 ppm	86/86	128/119	119/104
NR-421	3.6	88%	+14.2 (>100%)	+13.9 (>100%)	78/78	119/114	107/95
Mean of 3 checks	*4.1*	*100%*	*NA*	*NA*	*90/87*	*128/122*	*109/87*

[1] Mean of 3 checks across 2 stations (Islamabad, Faisalabad) during 2010/11 crop season

[2] Under optimal production conditions (5 irrigations) / moisture stress conditions (2 irrigations)

Strategic Factors Driving Delivery

Wheat is the staple food crop in Pakistan, grown on 8.63 million hectares with an annual production of 24.3 million tons. Despite its heavy consumption, the prevalence of zinc deficiency in children and women is common. The commercial wheat varieties are inherently low in zinc content, with an average of 25 ppm.

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Seed Commercialization

The biofortified wheat seed will be multiplied and marketed largely through public and private seed sectors, with participating companies encouraged to launch a special campaign for the variety. At the same time, federal and provincial governments will be approached to launch awareness campaigns through print and electronic media to promote the use of biofortified wheat flour for the reduction of malnutrition.

Marketing

Demand for zinc wheat is built through coordination with the Provincial Agriculture and Health departments, by educating household decision makers through extension agents, community health workers, and teachers, on the health benefits associated with zinc wheat. Building product acceptance is further facilitated by the agronomic superiority of the high-zinc lines in testing compared to currently grown varieties. Farmers will be educated on best cultivation practices for biofortified wheat through demonstration plots, fairs, meetings, and seminars.

Stakeholders

HarvestPlus collaborates with public and private sector, and nongovernmental organizations (NGOs), including the Ministry of Planning, Development and Reforms, Ministry of National Food Security and Research, Ministry of National Health Services, Regulation and Coordination, Provincial Food, Agriculture and Health departments, PARC, Federal Seed Certification and Registration Department, Food and Agriculture Organization of the United Nations (FAO), World Food Programme (WFP), UNICEF, United States Agency for International Development (USAID), UK Department for International Development (DFID), the farming community, Pakistan Flour Mills Association, and Pakistan Food Processors Association.

Potential Impact

It is projected that zinc wheat in Pakistan will enter the saturation stage of delivery by 2018, by which year the projected market share will be 1.5 percent. It is estimated that 250,000 farming households will have access to zinc wheat by 2018.

Cost

HarvestPlus will spend an estimated total of US$2.9 million for zinc wheat delivery activities in the period 2013–2018.

Delivery Challenges and Recommendations

- Great potential exists for biofortified zinc wheat production and consumption by farmers and consumers in Pakistan. This is attributable to the fact that the high-zinc trait is invisible and non-distinguishable. However, farmers will still need to be convinced to purchase the biofortified wheat. Extensive advocacy programs through electronic and print media will be required in the near future.
- At present, the wheat breeding program in Pakistan is exclusively in the public sector, and the private sector relies on public sector varieties, although this situation may change in the future. The HarvestPlus strategy entails engaging the private sector initially in testing of final products such as candidates for release. By gradually including germplasm at earlier development stages, the private sector can then establish zinc wheat testing programs and further develop intermediate-stage germplasm into their own zinc-dense final products as market opportunities emerge.

PROGRESS BRIEF #32

CROP DELIVERY

Delivery of Zinc Rice in Bangladesh

Md. Khairul Bashar & Md. Alamgir Hossain (CIAT-HarvestPlus)

Table 1. Staple Food Production & Consumption in Bangladesh

Staple Food	Daily Per Capita Consumption *(all age groups, grams/day)*[1]	Total Annual Production *(thousand metric tons)*[2]
Rice	438 g/d	33,889
Irish Potatoes	81 g/d	8,205
Wheat	44 g/d	995
	Daily Per Capita Consumption *(grams/day)*[3]	**Zinc Density**
Rice	Children 3–4 years: 169 g/d Women: 422 g/d	Conventional Rice: 16 parts per million (ppm) Zinc Target Increment: +12 ppm Biofortified Rice Target: 28 ppm At the target level, biofortified rice provides 75-90% of the Estimated Average Requirement (EAR).

[1]FAO Stat 2009; [2]FAO Stat 2012; [3]HarvestPlus Surveys

Table 2. Current Zinc Status

Prevalence of inadequate zinc intake (HarvestPlus study)	Children 2-4 years: 22% Women: 73%

Varietal Release

A number of zinc-rich rice advanced breeding lines for both *Boro* (irrigated) and *T. Aman* (rainfed) seasons are under development through a breeding program at the Bangladesh Rice Research Institute (BRRI). A high-zinc rice variety, BRRI dhan62, was recently approved by the National Seed Board of Bangladesh. It contains 19 ppm zinc and 9 percent protein, and yields 4.2 tons per hectare (similar to other popular, conventional varieties like BRRI dhan33 and BINA dhan7). In addition, BRRI dhan62 can be harvested within 100 days, which is the shortest duration *T. Aman* variety in the country (BINA dhan7 and BRRI dhan33 take 112–115 days). Due to its short duration, BRRI dhan62 can also escape terminal drought.

A multi-location trial was conducted with three zinc leading breeding lines in 80 locations across the country. The potential high-zinc lines had a comparable yield to popular variety BRRI dhan28, but zinc content was higher (22.8–25.5 mg/kg against 16.3 mg/kg for BRRI dhan28), close to 100 percent of the target level. Among these three lines, at least one will be released for cultivation in the *Boro* season.

Table 3. Average Performances of Zinc Rice in Multi-location Trials, Boro, 2013–2014

Designation	Plant Height (cm)	Growth Duration (days)	Grain Yield (t/ha)	Grain Zinc Content* (mg/kg)
BR7840-54-3-1	102	147	6.2	23.3 (+7.0)
BR7840-54-1-2-5	107	148	6.1	25.5 (+9.2)
BR7840-54-2-5-1	104	148	6.1	22.8 (+6.5)
BRRI dhan28 (check)	*100*	*145*	*6.2*	*16.3*

* Grain zinc content in polished rice was analyzed using XRF

Strategic Factors Driving Delivery

Rice is a widely consumed staple crop in Bangladesh. Seed and crop delivery is well established among private, governmental and nongovernmental agencies with which HarvestPlus collaborates. However, rice breeding is mainly through the public sector and is conducted by BRRI. In the absence of profitable commercial markets—rice is a notified crop in Bangladesh—the seed industry

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is reluctant to engage in breeding. Furthermore, it currently also lacks breeding capacity. This situation may change once hybrid rice becomes a commercial opportunity.

Seed Commercialization

HarvestPlus is capitalizing on existing seed networks and partnering with both public and private sector seed producers in zinc rice seed production and marketing. Currently, 12 nongovernmental organizations (NGOs) and two seed producer associations comprising 250 small- and medium-scale seed producers are involved in zinc rice seed production and delivery. Breeder seed production began in the 2013/14 *Boro* season. During 2013–14, 1,000 farm demonstrations and distribution of minikits (seed packets) got underway. Training of various rice delivery stakeholders in marketing and sales is also being facilitated by HarvestPlus.

Marketing

HarvestPlus will initially focus on seed production but, with increasing market presence after 2016, will initiate demand creation activities for zinc rice. Demand for zinc rice is created by educating household decision makers through extension agents, community health workers, and teachers, on the health benefits associated with zinc rice. Building product acceptance is further facilitated by the agronomic superiority of the high-zinc lines in testing compared to older varieties currently farmed. Village participatory selection trials will allow farmers to evaluate the attributes of candidate varieties under their own production conditions, and on-farm demonstration plots initiated in the 2013/14 *Boro* season will give farmers additional opportunities to observe zinc rice cultivation.

Stakeholders

HarvestPlus works closely with key government agencies such as the Seed Wing of the Ministry of Agriculture (MoA), BRRI and its SeedNet, Department of Agricultural Extension, and Bangladesh Agricultural Development Cooperation. Collaborators from the University of California-Davis and the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) carry out background food and nutrition surveys.

Potential Impact

By 2016, an estimated 500,000 households will be reached with zinc rice through commercialized seed sales. The long-term objective is to develop sustainable markets for zinc seed and grain, reaching a market share of over 3 percent of rice by 2018.

Cost

HarvestPlus will spend an estimated total of US$10 million for zinc rice delivery activities during the period 2013–2018.

Delivery Challenges and Recommendations

- According to government seed regulations, breeder seed can only be produced once official release is granted. This impedes bulking up of seed for a product launch after a variety is released.
- During milling, different varieties are usually bulked with paddy from similar grain types to produce a commercial grade of grain. This holds a potential risk of adulteration by combining other rice varieties with zinc rice.
- It is recommended to breed several zinc rice varieties for different growing seasons with popular attributes like higher yield, resistance to major diseases and pests, and consumers' preferred qualities to replace existing mega varieties for both *Boro* and *T. Aman* seasons.

PROGRESS BRIEF #33

CROP DELIVERY

Delivery of Iron Beans in Rwanda

Lister Katsvairo (CIAT-HarvestPlus)

Table 1. Staple Food Production & Consumption in Rwanda

Staple Food	**Daily Per Capita Consumption** *(all age groups, grams/day)*[1]	**Total Annual Production** *(thousand metric tons)*[2]
Beans	80 g/d	433
Maize	46 g/d	573
Cassava	258 g/d	2,716
Irish potatoes	282 g/d	2,337
Sweet potatoes	200 g/d	1,005
	Daily Per Capita Consumption *(grams/day)*[3]	**Iron Density and Iron Intakes**
Beans	Children (3-5 years): 65 g/d Women: 123 g/d	Conventional Bean: 50 parts per million (ppm) Iron Target Increment: +44 ppm Biofortified Bean Target: 94 ppm
		At the target level, biofortified beans provide about 60% of the Estimated Average Requirement (EAR).

[1]FAO Stat 2009; [2]FAO Stat 2012 [3]HarvestPlus Surveys

Table 2. Current Iron Status

Prevalence of anemia (2010 DHS)	Children under five: 38% Women: 17%

Varietal Release

To date, four first-wave varieties with more than 60 percent of the iron level and five second-wave varieties with 60–94 percent of the target have been released in Rwanda, as detailed in the chart below. In 2013, HarvestPlus began working with private farmers, cooperatives, and nongovernmental organization (NGO) partners to produce certified seed and expand seed production and marketing to up-and-coming seed companies. Currently, 500 metric tons of certified seed is produced per year through 80 registered seed multipliers.

Strategic Factors Driving Delivery

Building product acceptance is facilitated by the agronomic superiority of recently released high-iron varieties compared to older varieties currently farmed. Average bean yields in Rwanda of nonbiofortified beans are approximately 0.8 tons/hectare (bush and climbers combined); biofortified bush beans yield around 1.5 t/ha and biofortified climber beans 2–3 t/ha on farm. Furthermore, awareness and demand for iron seeds are created by educating household decision makers on the health benefits associated with consuming iron beans.

Seed Commercialization

Distribution channels for iron beans include direct marketing (mobile sales), agrodealers, cooperatives, and a payback system. For direct marketing, seed is packed into small packages of 500 grams and 1 kilogram and sold to farmers on market days. Agrodealers (farm input suppliers) are used to distribute the same sizes of small packs to farmers. Cooperatives use their organized structures to distribute seed to their farmers. In addition to seed sales, an experimental payback system was implemented in six districts of the country (Bugesera, Gatsibo, Kayonza, Kamonyi, Nyanza, and Huye) where agrodealers are not present. In this approach, governmental structures are used to distribute seed to farmers on a larger scale with larger quantities. This approach increases farmers' ability to reach household consumption levels faster than with the sales approach, as farmers often have low levels of disposable income. The distribution partners are also trained in promotion and nutrition messaging.

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Table 3. Iron Bean Varieties Released in Rwanda

Name	Source	Iron Content[1]	% Target level	Agronomic Characteristics				
				Seed Color	Adaptation	Resistance[2]	Tolerance[2]	Yield Potential (t/ha)
First Wave (fast-track): 50–60% target increment, release in 2010								
RWR 2245 (Bush)	RAB	76 ppm	59%	Red mottled	Low to mid altitude	AB, AC, BCMV	ALS, RR	2.5
RWR 2154 (Bush)	RAB	71 ppm	47%	Sugar	Low to mid altitude	AB, AC, BCMV	ALS	2.0
MAC 44 (Climber)	CIAT	78 ppm	64%	Red mottled	Mid to high altitude	AC	AB,ALS, BCMV, RR	3.5
RWV 1129 (Climber)	RAB	77 ppm	61%	Salmon	Mid to high altitude	AC, BCMV, RR	AB,ALS	3.0
Second wave: 80–90% target increment, released in 2012								
RWV 3006	RAB	78 ppm	63%	White	Mid to high altitude	AB, AC, ALS, BCMV	-	3.8
RWV 3316	RAB	87 ppm	84%	Red	High altitude	AC, BCMV	AB,ALS	4.0
RWV 3317	RAB	74 ppm	54%	Sugar	High altitude	AC, BCMV	AB,ALS	4.0
MAC 42	CIAT	91 ppm	94%	Sugar	Low to mid altitude	AC, BCMV	AB,ALS	3.5
RWV 2887	RAB	85 ppm	80%	Dark red	High altitude	AC, BCMV	AB,ALS	3.8

[1] Average across 4 seasons, ICP and XRF data

[2] AB: Ascochyta blight; AC: Anthracnose; ALS: Angular leaf spot; BCMV: Bean common mosaic virus, RR: Root rot

Marketing

HarvestPlus initially focused on seed sales. With increasing market presence, HarvestPlus has now initiated demand creation for iron bean grain. Significant volumes of iron bean grain are appearing in markets in urban centers, an indication of the saturation effect noted in certain farming districts. Test markets are being used to generate diagnostic information, allowing for revision of the marketing plan. This includes testing of messages and product benefits, communication channels, brand name and specific promotional messages, activities, and advertising that resonate best with the consumers.

Stakeholders

Partnerships are extremely important to the delivery efforts in Rwanda, and HarvestPlus works closely with the Rwanda Agriculture Board (RAB), emerging private sector seed companies, agrodealers, cooperatives, and traders. Biofortification is included in Government of Rwanda policies, including in the Ministry of Agriculture's Nutrition Action Plan.

Potential Impact

At the end of 2013, a cumulative 714,000 farming households in Rwanda had been reached with iron bean seed. HarvestPlus plans to reach about 1.2 million farming households - virtually all bean-growing farm households - in the country by 2016,. Biofortified beans will reach a market share greater than 50 percent by 2018 and will be accessible to nonproducing households in urban and rural areas.

Cost

HarvestPlus will spend an estimated total of US$2.8 million for iron bean delivery activities in the period 2011–2016.

Delivery Challenges and Recommendations

- Quantities of basic and foundation seed remain a challenge; HarvestPlus is exploring renting additional land on which to produce seed while working hand-in-hand with RAB.
- Most farmers typically purchase grain to use as seed; purchasing iron bean seed requires a change in mindset.
- Different marketing approaches are needed to increase demand for iron bean seed and iron bean grain; to date HarvestPlus has primarily focused on seed demand.
- HarvestPlus seeks to first improve the nutritional status of farming families, but many iron bean varieties are attractive for marketing and export.

Delivery of Iron Beans in Democratic Republic of Congo (DRC)

Antoine Lubobo (CIAT-HarvestPlus)

Table 1. Staple Food Production & Consumption in DRC

Staple Food	**Daily Per Capita Consumption** *(all age groups, grams/day)*[1]	**Total Annual Production** *(thousand metric tons)*[2]
Cassava	Data not available for DRC	16,000
Maize		1,200
Rice		350
Sweet Potato		265
	Daily Per Capita Consumption *(grams/day)*[3]	**Iron Density and Iron Intakes**
Beans	Children (3-5 years): 65 g/d Women: 123 g/d *From Rwanda	Conventional Bean: 50 parts per million (ppm) Iron Target Increment: +44 ppm Biofortified Bean Target: 94 ppm
		At the target level, biofortified beans provide about 60% of the Estimated Average Requirement (EAR).

[1]FAO Stat 2009; [2]FAO Stat 2012 [3]HarvestPlus Surveys

Table 2. Current Iron Status

Prevalence of anemia (2007 DHS)	Children 6-59 months: 71% Women: 53%

Varietal Release

To date, 10 first- and second-wave varieties with up to 100 percent of the iron target have been disseminated to more than 150,000 households in the Democratic Republic of Congo (DRC). Three fast-track varieties (2 bush, 1 climber) were released in DRC in 2008, one bush bean was released in 2011, and one climber variety was released in 2012. These varieties were identified for first-wave seed multiplication and dissemination. Five second-wave varieties with 60–100 percent of the iron target were released in 2013. Varieties combine competitive yield with farmer-preferred end-use quality, and cover major market classes. Seed dissemination channels include direct marketing (mobile sales), farmers' associations/community-based organizations (CBOs), and nongovernmental organizations (NGOs). Delivery partners are also trained in promotion. The table below presents varieties released in DRC.

Strategic Factors Driving Delivery

Awareness and demand for iron seeds are created by educating household decision makers on the health benefits associated with consuming iron beans. Building product acceptance is further facilitated by the agronomic superiority of recently released iron varieties compared to older varieties currently farmed. Beans are prominent in the diets of people in eastern DRC.

Seed Commercialization

Initially, demo seed packs were offered for free to farmers. Based on this experience, seed dissemination was scaled up for sale at either market or subsidized prices. In 2012 and 2013, 25 percent of bean packs were distributed for free (in the area with insecurity) and 75 percent sold. Beans were distributed by 31 partners in four provinces. The pack size distribution percentage was approximately 50, 30, 20 for 250 gram, 500 gram and 1 kilogram packages, respectively. These proportions may change in future based on delivery experience. Different seed pack sizes are required for different regions of the country, with Eastern DRC requiring small packs of 250 grams to 5 kilograms and other regions requiring larger packs of 10–50 kilograms. Two high-value varieties have been identified, and these will be targeted for seed commercialization through agrodealer networks, merchandisers and mobile sales.

Table 3. Iron Bean Varieties Released in DRC

Variety Name	Release Year	Country	Iron Content* (% target)	Altitude Range; Color; Disease Reaction
First Wave (fast-track): 50–60% target increment				
RWR 2245 (Bush)	2011	DRC	76 ppm (59%)	Low to mid altitude; color red mottled; AB, AC resistance; ALS, RR tolerance
COD MLB 001 (Bush)	2008	DRC	64 ppm (32%)	Low to mid altitude; color red mottled; AB, AC resistance; ALS, RR, drought tolerance
Hm 21-7 (Bush)	2008	DRC	62 ppm (27%)	Low to mid altitude; color red mottled; AB, AC, RR resistance; ALS, drought tolerance
COD MLV 059 (Climber)	2012	DRC	84 ppm (77%)	Mid to high altitude; color red mottled; AC, CBB, RR resistance; ALS tolerance
VCB 81013 (Climber)	2008	DRC	69 ppm (43%)	Mid to high altitude; color white; AC, CBB, RR resistance; ALS tolerance
Second Wave: 80–90% target increment				
PIGEON VERT (Bush)	2013	DRC	80 ppm (68%)	Low to mid altitude; color yellow; AC, BSM, CBB, RR resistance; LSF, drought tolerance
PVA 1438 (Bush)	2013	DRC	79 ppm (66%)	Mid to high altitude; color red kidney; CBB, RR resistance
COD MLB 032 (Bush)	2013	DRC	76 ppm (60%)	Mid to high altitude; color sugar; AB, AC resistance; ALS, RR, drought tolerance
CUARENTINO (Climber)	2013	DRC	100 ppm (114%)	Mid to high altitude; color white; AC, CBB resistance; RR tolerance
NAIN DE KYONDO (Climber)	2013	DRC	76 ppm (60%)	Mid to high altitude; color white; ALS, RR resistance; AB tolerance

*Average across four seasons, ICP and XRF data.

Notes: AB: Ascochyta blight; AC: Anthracnose; ALS: Angular leaf spot; BCMV: Bean common mosaic virus; RR: Root rot

Marketing

HarvestPlus initially focused on seed sales. With increasing market presence, HarvestPlus has now initiated demand creation for iron bean grain. Test markets are used to generate diagnostic information, allowing for revision of the marketing plan prior to national rollout. This includes testing which messages, product benefits, communication channels, brand names and specific promotional messages, activities, and advertising resonate best with the consumers.

Stakeholders

Partnerships are extremely important to the delivery efforts in DRC, and HarvestPlus works closely with DRC's national agricultural research organization (INERA), the DRC National Seed Services (SENASEM), extension services, farmers' associations/CBOs, and several NGOs.

Potential Impact

At the end of 2013, a cumulative 150,000 farming households in DRC had been reached with iron bean seed. HarvestPlus plans to develop sustainable markets for seed and grain, and reach a market share greater than 45 percent for iron beans by 2018. An estimated 1,375,000 farming households in DRC will have access to iron beans by 2014.

Cost

HarvestPlus will spend an estimated total of US$2.5 million for bean delivery activities in the period 2013–2018.

Delivery Challenges and Recommendations

- Most farmers typically purchase grain to use as seed; purchasing iron bean seed requires a change in mindset.
- Different marketing approaches are needed to increase demand for iron bean seed and iron bean grain; HarvestPlus has primarily focused on seed demand to date.
- There is need to build and strengthen alliances on sustainable seed systems with key players including government, NGOs, the Food and Agriculture Organization of the United Nations (FAO), and the World Food Programme (WFP) to recognize the agronomic and nutrition value of iron beans.

PROGRESS BRIEF #35

CROP DELIVERY

Delivery of Vitamin A Cassava in Nigeria

Paul Ilona (CIAT-HarvestPlus)

Table 1. Staple Food Production & Consumption in Nigeria

Staple Food	Daily Per Capita Consumption *(all age groups, grams/day)*[1]	Total Annual Production *(thousand metric tons)*[2]
Cassava	281 g/d	54,000
Maize	81 g/d	9,410
Millet	95 g/d	5,000
Rice	57 g/d	4,833
	Daily Per Capita Consumption *(grams/day)*[3]	**Provitamin A Density**
Cassava	Children (2-5 years): 350 g/d Women: 900 g/d	White Cassava: 0 parts per million (ppm) Provitamin A Target Increment: +15 ppm Biofortified Cassava Target: 15 ppm
		At the target level, biofortified cassava provides about 50% of the Estimated Average Requirement (EAR).

[1]FAO Stat 2009; [2]FAO Stat 2012; [3]HarvestPlus Surveys

Table 2. Current Vitamin A Status

Prevalence of vitamin A deficiency (HarvestPlus Survey)	Children under five: 25%

Varietal Release

Three first-wave vitamin A cassava varieties with 50–60 percent of the target level were officially released in December 2011. Second-wave varieties with 70–80 percent of the full target level were expected to be released in 2013/14 and full target varieties in 2015/16. More than 50 vitamin A leads are now at different stages of evaluation to identify those that are agronomically competitive for the third wave. These varieties will be put in tissue culture for international distribution, particularly targeting potential expansion countries.

Vitamin A maize and orange sweet potato (OSP) have also been officially released in Nigeria while the introduction of other biofortified crops like iron pearl millet, iron bean, and zinc rice has been planned for the future.

Table 3. First-Wave Vitamin A Cassava Varieties Released in Nigeria in 2011 (50–60% target increment)

Variety Name	Total Carotenoid Content (FW)*	Fresh root yield	Yield Relative to Check	Dry matter
TMS 01/1371	7.8 ppm	20.1 t/ha	87%	30.7%
TMS 01/1412	7.4 ppm	29.8 t/ha	128%	30.1%
TMS 01/1368	6.9 ppm	26.7 t/ha	115%	33.4%
30572 (Check)	*0.9 ppm*	*23.2 t/ha*	*100%*	*37.1%*

*Provitamin A content is approximately 80% of Total Carotenoid Content (fresh weight)

Delivery Strategy

HarvestPlus builds on existing extension pathways originally designed for other cassava projects funded by the World Bank and the International Fund for Agricultural Development in Nigeria. In addition to providing technical assistance to strengthening the different value chain operators, the delivery strategy empowers the downstream population where hidden hunger is a challenge to sustainably scale up production and processing of vitamin A cassava to meet both food and income needs. Four states (Oyo – West, Imo – East, Akwa Ibom – South, and Benue – North) are initially targeted and will be used as hubs to reach all other states.

Seed Multiplication

HarvestPlus works with farmers, stem traders, cooperatives, government extension, and nongovernmental organization (NGO) partners to multiply stems. In 2013, over 650 hectares of released vitamin A cassava varieties were multiplied in 272 villages. Over 1,000 hectares will be multiplied in 2014. Private sector engagement increased from 5 percent in 2012 to 32 percent in 2013 and is expected to account for over 70 percent of total stem production and trade by 2018. Using improved agronomic practices, average stem yield on multiplication farms increased from 200 to 500 bundles on-farm and to 1,000 on-station.

Seed Delivery

In 2013, over 106,000 farmers received and planted vitamin A cassava stems in four target and 10 expansion states. This number will grow three-fold in 2014, exceeding 350,000 farmers as more partners engage in stem dissemination to vulnerable households. Women represented 45 percent of the recipients of stems in 2013 but may account for over 60 percent by 2016.

Value Addition

Twenty-five traditional meal and confectionery food products have been developed using vitamin A cassava and are documented in a recipe book to be published in 2014. Ten other innovative foods and beverages are in the final stages of evaluation for commercial processors and marketers. This will create and diversify markets for vitamin A cassava for sustainable adoption.

Marketing

On average, rural farmers consume 40 percent and sell 60 percent of their cassava. In 2013, almost 90 percent of the estimated 7,000 tons of vitamin A cassava roots harvested from multiplication farms was consumed while only 10 percent was sold. As farmers adopt and increase the production of vitamin A cassava on their farms, it is expected that the ratio of products sold will progressively increase to its maximum depending on the market.

Consumer direct marketing using radio, television, and print media, as well as cellphone short text messaging (SMS) and farmer field days, has been used to educate Nigerians on the importance of consuming more nutritious foods and for communicating supply and demand information. It is estimated that over 30 million Nigerians have already received information on biofortification with an emphasis on vitamin A cassava.

With increasing market presence, HarvestPlus has initiated demand creation for yellow cassava tubers and its products such as *gari* and *fufu*. Groups of processors that have a commercial focus have been identified in each of the target states to process fresh yellow roots into *gari* and *fufu* for sale. There is growing demand for vitamin A *gari* by Nigerians in the diaspora, and 40 tons were exported to Europe in October and November 2013 by Niji Lukas, a private food company based in Oyo State, Nigeria.

Stakeholders

Partnerships are extremely important to the delivery efforts in Nigeria. HarvestPlus works closely with the ministries of Agriculture and Health, International Institute of Tropical Agriculture (IITA), the National Root Crops Research Institute (NRCRI), NGOs, universities, and food companies. The Federal Ministry of Agriculture and Rural Development has continued to provide both political and financial support for the development and dissemination of biofortified food crops, which are fully integrated into the Agricultural Transformation Agenda (ATA) of the Federal Government. The Ministry of Health has also provided support by including biofortified cassava, maize, sweet potato, and pearl millet in the new Micronutrient Deficiency Control Guidelines that were approved by the National Health Council in August 2013.

Potential Impact

Nigeria has a huge cassava market, producing over 54 million metric tons annually, engaging over four million farmers in production, and providing food for over 100 million people. At the end of 2013, a cumulative 106,000 farming households had been reached with vitamin A cassava. It is estimated that by 2018 more than two million farming households will be planting vitamin A cassava and at least 17 million rural and urban consumers will be eating vitamin A *gari* and *fufu* in their regular diets.

Cost: HarvestPlus will spend an estimated total of US$10 million on cassava delivery activities in the period 2013–2018.

Delivery Challenges and Recommendations

- Only a limited number of national breeders focus on cassava, which may be a constraint in the future. HarvestPlus will ensure that national breeders are further supported, their capacities strengthened and updated to respond to advances in breeding for high nutrient levels in crops.
- Increasing trends in disease and pest pressures, climate change, and soil degradation may limit the supply of vitamin A cassava, depending on how current varieties respond to the changing environment. This suggests that more robust varieties need to be continuously developed in the years ahead.
- Cassava has a low multiplication ratio, often not exceeding 1:10 in conventional multiplication and only 1:5 in rural on-farm situations. By using improved multiplication and agronomic practices, the multiplication ratio can increase to 1:30.
- The target population is multi-sectorial and multi-cultural. As a result, HarvestPlus has embraced and created a mechanism for all sectors, both public and private, to participate. The delivery process is cost intensive at the initial stage so governments at all levels are encouraged to invest in the development and dissemination of nutritious crops. A supportive policy would be allocating a portion of the national agricultural budget to support the development of value chains for more nutritious crops.

PROGRESS BRIEF #36

CROP DELIVERY

Delivery of Vitamin A Cassava in Democratic Republic of Congo (DRC)

Sylvain Bidiaka (CIAT-HarvestPlus)

Table 1. Staple Food Production & Consumption in DRC

Staple Food	**Daily Per Capita Consumption** *(all age groups, grams/day)*[1]	**Total Annual Production** *(thousand metric tons)*[2]
Cassava	1,240 g/d (fresh weight)	16,000
Maize	Data not available for DRC	1,200
Rice		350
Sweet Potato		265
	Daily Per Capita Consumption *(grams/day)*[3]	**Provitamin A Density**
Cassava	Children (4-6 years): 200 g/d Women: 400 g/d	White Cassava: 0 parts per million (ppm) Provitamin A Target Increment: +15 ppm Biofortified Cassava Target: 15 ppm
		At the target level, biofortified cassava provides about 50% of the Estimated Average Requirement (EAR)

[1]FAO 2000; [2]FAO Stat 2012; [3]Estimated

Table 2. Current Vitamin A Status

Prevalence of vitamin A deficiency in Study Area (1)	Children under five: >50%

Varietal Release

One variety with 70 percent of the provitamin A target, released in DRC in 2008, was selected for first-wave dissemination. Stem multiplication began in late 2011, and planting materials were disseminated to 25,000 farming households in 2013, initially focusing on four provinces: Kinshasa, Bas-Congo, Orientale, and Kivu. Second-wave varieties with the full target level for high per capita consumption areas are expected to be fast-tracked for release by 2014.

Table 3. Fast-track Variety Released in 2008 (First Wave), 70% target increment

Variety Name	Origin	Total Carotenoid Content (FW)*	Provitamin A Content (FW)*	Fresh Root Yield	Dry Matter
I011661	IITA (Nigeria)	9.4 ppm	7.6 ppm	34.9 t/ha	30%
Butamu (Check)	IITA (DRC)/INERA	4.4 ppm	3.9 ppm	35.0 t/ha	35%

* Provitamin A content is approximately 80% of Total Carotenoid Content (fresh weight – FW); measured with spectrophotometer.

Strategic Factors Driving Delivery

As in Nigeria, the adoption of vitamin A cassava is expected to be driven by the demand for its processed products like *gari*, *chikwangue,* and *fufu*. Promotion plans are, therefore, designed to increase consumption of these processed products toward achieving food security and improved income. The cassava market is still largely informal, with markets for cassava stems and processed products just emerging. However, this situation is changing as small and medium enterprises (SME) in cassava processing are entering the market.

Seed Commercialization

Stem dissemination channels include the National Extension Service (SNV), direct marketing, nongovernmental organizations (NGOs), and farmers' associations. Partners are also trained in promotion. The National Seed Service (SENASEM) is in charge of seed control and certification in the multiplication fields and approves the quality of planting materials being disseminated, while

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the SNV assists in identifying beneficiary households and farmers. NGOs and farmers' associations have been identified in the selected provinces for stem multiplication at secondary levels and distribution of planting materials to individual and group farmers. Other actors in the vitamin A cassava market in DRC include: (1) low-volume traders who specialize in marketing cassava stems and products within short distances, mostly within villages; and (2) higher-volume wholesalers who buy and warehouse stems and products before selling them in urban or distant markets.

Marketing

Awareness and demand for yellow cassava are created by educating household decision makers on the health benefits associated with consuming vitamin A cassava. HarvestPlus initially focused on stem production. With increasing market presence after 2013, HarvestPlus is initiating demand creation for yellow cassava tuberous roots and products. Market testing examines which messages and product benefits, communication channels, brand names and promotional messages, activities, and advertising best resonate with consumers. Trader and retailer product incentives for segregated display of yellow and white cassava will be examined, and lessons learned from the promotion of vitamin A cassava in Nigeria will be applied in DRC.

Stakeholders

Partnerships are extremely important to the delivery efforts in DRC, and HarvestPlus works closely with the Ministry of Agriculture (national and provincial levels) and the Ministry of Health (through PRONANUT, the National Nutrition Program), National Agriculture Research System (NARS) and extension services, and the Food and Agriculture Organization of the United Nations (FAO), as well as a number of local NGOs and farmers' associations.

Potential Impact

At the end of 2013, a cumulative 25,000 farming households in DRC had been reached with vitamin A cassava. The long-term objective is to develop sustainable markets for stems and tuberous roots, develop a long-term brand for tuberous roots, and integrate vitamin A cassava into NARS/extension and school feeding programs. The goal is to reach a cassava market share greater than 7 percent by 2018, with 750,000 farming households having access to vitamin A cassava.

Cost

HarvestPlus will spend an estimated total of US$1.05 million for cassava delivery activities in the period 2013–2018.

Delivery Challenges and Recommendations

- Significant increases in farmers' and consumers' awareness of the benefits of vitamin A cassava is critical to success.
- While HarvestPlus-DRC has focused largely on varietal development and stem/seed multiplication in its initial years of operation, advocacy efforts are a critical component to address in the next phase.
- The branded market for cassava products in DRC is essentially unknown due to culture, tradition, and income limitations. Therefore, HarvestPlus' emphasis will be on finding more tactical yet creative sales approaches for vendors of these products to differentiate them in the marketplace.
- Further encouragement of private sector participation in multiplication, processing, and marketing of products is needed.

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1. Samba, C; Gourmel, B; Houze, P; Malvy, D. 2010. Assessment of vitamin A status of preschool children in a sub-Saharan African setting: Comparative advantage of modified relative-dose response test. *Journal of Health Population and Nutrition* 28(5):484–493.

PROGRESS BRIEF #37

CROP DELIVERY

Delivery of Vitamin A Maize in Zambia

Eliab Simpungwe (CIAT-HarvestPlus)

Table 1. Staple Food Production & Consumption in Zambia

Staple Food	Daily Per Capita Consumption *(all age groups, grams/day)*[1]	Total Annual Production *(thousand metric tons)*[2]
Maize	302 g/d	2,853
Cassava	237 g/d	1,300
	Daily Per Capita Consumption *(grams/day)*[2]	**Provitamin A Density**
Maize	Children (2-5 years): 172 g/d Women: 287 g/d	White Maize: 0 parts per million (ppm) Provitamin A Target Increment: +15 ppm Biofortified Maize Target: 15 ppm
		At the target level, biofortified maize provides about 50% of the Estimated Average Requirement (EAR).

[1]FAO Stat 2009; [2]FAO Stat 2012; [3]HarvestPlus Surveys

Table 2. Current Vitamin A Status

Prevalence of vitamin A deficiency (NFNC 2003)	Children 6-59 months: 53%

Varietal Release

After years of plant breeding research, the first wave of orange-colored vitamin A maize hybrid varieties, which provide 50 percent of the full target provitamin A level, were released in September 2012. As a precursor to the release of orange maize, 500 demonstration plots were planted by lead farmers in November 2011, intended to create awareness and allow farmers to evaluate the varieties. In November 2012, close to 1,000 lead farmers planted orange maize in plots large enough to feed a family of five over six months. Future breeding efforts focus on developing higher yielding, more robust hybrids exploiting specific adaptation to the different agroecological zones in Zambia.

Table 3. First Wave Varieties of Maize Released in 2012 in Zambia

Variety Name	Release Year	Vitamin A Content	Comments on Agronomic Properties
First Wave (fast-track): 6–8 ppm Vitamin A Content			
GV662A	2012	7 ppm	Medium maturity hybrid of medium tall plant stature; semi-flint grain, good resistance to GLS and turcicum blight; highly ranked in farmer preference; yellow orange grain; competitive yielder
GV664A	2012	7 ppm	Medium maturity hybrid of medium tall plant stature; semi-flint grain, good resistance to GLS and turcicum blight; highly ranked in farmer preference; orange grain; competitive yielder
GV665A	2012	8 ppm	Medium maturity hybrid of medium tall plant stature; flint grain; good resistance to GLS and turcicum blight; highly ranked in farmer preference; orange grain; competitive yielder; double cobbing tendency

Notes: GLS: Gray leaf spot

Strategic Factors Driving Delivery

Maize is the primary staple food crop consumed in Zambia and is by far the most important agricultural crop, allowing significant potential for market share. The inherent nutritional superiority of orange maize is a compelling product benefit.

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Seed Commercialization

The current seed market for maize hybrids in Zambia is estimated at 20,000 tons per year and is projected to increase to 22,000 tons by 2018. Achieving a 10 percent market share for orange maize seed by 2018 constitutes the minimum HarvestPlus target. To ensure long-term sustainability and competitiveness, seed companies have plans to engage in vitamin A maize breeding, thereby establishing their own vitamin A maize product lines. Seed is produced by seed companies, which employ experienced seed growers in production. Training in seed multiplication is conducted within companies. HarvestPlus will partner with private seed companies for quality seed production, sales, and marketing of vitamin A maize varieties, while continuing its partnerships with public institutions.

Marketing

Creating demand for orange maize through nutrition information dissemination and market stimulation for grain constitutes the main thrusts of the marketing campaigns. As studies have shown that nutrition campaigns can translate into improved acceptance and willingness to pay for orange maize, information dissemination on the comparative advantage of orange maize will be a major marketing campaign initiative. Print media and radio (both local community and national radio) are being used throughout the country and, on a preliminary basis, look effective. The national and regional agricultural shows have been used as promotional platforms. Overall, HarvestPlus marketing activities are complementary to private sector marketing campaigns; seed companies and processors undertake their own marketing campaigns whenever they are introducing a new product on the market.

Stakeholders

Partnerships are extremely important to the delivery efforts in Zambia, and HarvestPlus works closely with the Zambia Agriculture Research Institute (ZARI), World Bank AgResults project, Zambia Ministry of Health and several NGOs, among others. A partnership is in the pipeline with the World Food Programme Purchase-for-Progress program (WFP-P4P) to supply orange maize grain to school feeding programs in the future.

Potential Impact

At the end of 2013, a cumulative 10,000 farming households in Zambia had been reached with vitamin A maize. It is projected that vitamin A maize in Zambia will be transitioning from the saturation to the anchoring stage of delivery by 2018. The projected market share by 2018 will be 10 percent; it is projected that approximately 500,000 farming households will have access to vitamin A maize.

Cost

HarvestPlus will spend an estimated total of US$5.3 million for maize delivery activities in the period 2013–2018.

Delivery Challenges and Recommendations

- The partnership with private seed companies to commercialize orange maize seed has the advantage of creating sustainability in the seed supply system but also the challenge of remaining dependent on their performance in meeting farmers' seed needs.

- The color barrier, whereby consumers prefer white maize over yellow and orange maize, is being surmounted with aggressive nutrition information dissemination. Acceptance studies indicated that once growers and consumers appreciate the nutrition benefits of orange maize, they place a premium on the crop above the white varieties.

Delivery of Biofortified Food Basket in Latin America & the Caribbean

Marilia Nutti (EMBRAPA-HarvestPlus)

Current Status

Because most Latin American and Caribbean (LAC) countries do not consume large amounts of a single staple crop, a different approach must be used in biofortification: a food basket. Elements of the food basket include iron beans, zinc rice, vitamin A cassava, vitamin A and zinc maize, and orange sweet potato (OSP). Supplementation and fortification programs in LAC are strong and reach much of the population, so HarvestPlus-LAC focuses particularly on reaching the rural poor—who may not have access to these other complementary interventions—with the biofortified food basket.

Breeding biofortified crops for LAC was previously coordinated by AgroSalud, based at the International Center for Tropical Agriculture (CIAT), but has now been integrated into the HarvestPlus portfolio. Additionally, the Government of Brazil supports BioFORT Brazil, coordinated by Embrapa, which focuses efforts on the poorest areas of northeast Brazil and aims to improve nutrition through school feeding programs.

Table 1. Biofortified Crops in Brazil

Crop	Variety Name	Iron (Fe) Content	Zinc (Zn) Content	Provitamin A Content	Content in Conventional Cultivars
Maize	BRS 4104	-	-	5-8 ppm	2-4 ppm provitamin A
Sweet potato	Beauregard	-	-	90-140 ppm	0-10 ppm provitamin A
Pumpkin	On-going research	-	-	140-240 ppm	20-60 ppm provitamin A
Wheat	On-going research	40-50 ppm	40-50 ppm	-	25-35 ppm Fe; 30-40 ppm Zn
Cowpea	BRS Xiquexique, BRS Tumucumaque, BRS Aracê	50-70 ppm	40-50 ppm	-	40-50 ppm Fe; 30-40 ppm Zn
Cassava	BRS Jari, BRS Gema de Ovo, BRS Dourada	-	-	4-9 ppm	0 ppm provitamin A
Common Bean	BRS Pontal, BRS Agreste, BRS Cometa	70-90 ppm	35-50 ppm	-	25-65 ppm Fe; 10-35 ppm Zn
Rice	On-going research	2-5 ppm	15-20 ppm	-	0.5-2 ppm Fe; 5-12 ppm Zn

*Parts per million (ppm)

Varietal Release

BioFORT Brazil has been working on biofortification for more than 10 years and has released several biofortified varieties of maize, sweet potato, cowpeas, cassava, and beans. Additional research is ongoing for wheat and rice. The following table presents the varieties that have been released or are in testing, compared against their conventional counterparts.

In other LAC countries, varietal releases to date include iron beans in El Salvador, Panama, Nicaragua, and Guatemala. Additionally, promising varieties of zinc rice are being tested in Bolivia, Panama, and Nicaragua. Going forward, AgroSalud will focus its attention on three priority countries—Guatemala, Haiti, and Nicaragua—where women and children suffer from the highest levels of vitamin and mineral deficiencies in the region.

Seed Distribution

BioFORT Brazil uses demonstration plots, organized through the national extension system, to provide smallholder farming households with seeds and stems of biofortified crops. Distribution in Panama is just getting underway, and HarvestPlus is helping to strengthen the seed system to increase rates of seed multiplication.

Table 2. Biofortified Beans Released in LAC

Variety Name	Country	Iron Content* (% target)	Zinc Content* (% target)
CENTA FERROMÁS	El Salvador	75-80 ppm (80-85%)	35 ppm (71%)
NUA 24	Panama	77 ppm (82%)	30 ppm (61%)
NUA 296	Panama	88 ppm (94%)	32 ppm (65%)
INTA Nutritivo	Nicaragua	63 ppm (67%)	28 ppm (57%)
Superchiva	Guatemala	59 ppm (63%)	31 ppm (63%)

* Measured by XRF

Seed Commercialization

HarvestPlus is capitalizing on existing seed networks and partnering with both public and private sector seed producers in zinc rice seed production and marketing. Currently, 12 nongovernmental organizations (NGOs) and two seed producer associations comprising 250 small- and medium-scale seed producers are involved in zinc rice seed production and delivery. Breeder seed production began in the 2013/14 *Boro* season. During 2013–14, 1,000 farm demonstrations and distribution of minikits (seed packets) got underway. Training of various rice delivery stakeholders in marketing and sales is also being facilitated by HarvestPlus.

Marketing

In Colombia, collaboration with the private sector offers a market for farmers' biofortified crops, which are then processed into typical Colombian products, such as *mazamorra* and *natilla*, made from biofortified maize, as well as zinc rice noodles and vitamin A cassava and sweet potato flour. In Brazil, as part of a school feeding pilot project, 10 cities have added iron beans, vitamin A cassava, and OSP to their programs, ensuring that small-scale farmers who grow biofortified crops have a market for their excess production.

Stakeholders

Partnerships are extremely important to the delivery efforts in LAC. They range from public sector partnerships, such as with the governments of Brazil and Panama that have created and support national biofortification programs, to private sector partnerships, such as with Pampa Ltd. Stakeholder workshops were held in late 2013 in Guatemala and Nicaragua; both countries have established national committees for the inclusion of biofortification in food security policies.

Potential Impact

From 2009 to 2013, nearly 2,000 farming households were reached with biofortified crops across five states in Brazil. In the states of Bahia, Maranhão, Minas Gerais, Piauí, Rio de Janeiro, and Sergipe, about 30 schools are including biofortified foods in their menus, benefitting more than 5,000 children around the country. The state of Piauí contains the largest number of partnerships. The partnerships are developed in conjunction with Agricultural Family Schools (EFAs), technical assistance companies, and municipal governments. They serve as a model of *Productive Safety*, a set of measures needed to reduce the risks of production losses and enable small farmers to produce their own food with guaranteed harvest.

Delivery Challenges and Recommendations

- Coordination of research and delivery across numerous countries can be difficult and unfocused as approaches differ by country.
- Nutrition evidence is not complete for varieties developed under AgroSalud.
- In Phase III, HarvestPlus will increase its work in LAC, focusing on developing a biofortified food basket for Guatemala, Haiti, and Nicaragua.
- HarvestPlus LAC will also develop a more complete portfolio of evidence around nutrition and adoption.

PROGRESS BRIEF #39

CROP DELIVERY

Biofortification in China

Xingen Lei (Cornell University)

Current Status

Biofortification was first introduced in China in 2004. After a decade-long effort by over 100 domestic scientists and international collaborators, the national biofortification program, called HarvestPlus-China, has met initial success. A total of nine interdisciplinary teams have been formed to focus on enriching rice, wheat, maize, and sweet potato with bioavailable iron, zinc, and vitamin A. Since 2004, 18 enriched lines have been developed, and eight of these lines (1 wheat, 1 rice, 1 maize, and 5 sweet potato varieties) have been approved for field dissemination. One additional maize line is in the final stages of approval. A human trial has been completed to determine the efficacy of a beta-carotene orange sweet potato (OSP) line in improving vitamin A status of school children. Two cost-effectiveness and impact analyses have shown significant public health benefit and economic gain from biofortification of sweet potato and wheat.

Table 1. Biofortified Crop Varieties Released in China

Crop and Variety Name	Release Year	Micronutrient Content *(parts per million – ppm)*	Comments on Agronomic Properties
Wheat – Zhongmai 175*	2008, 2010	Fe: 30–44.5 ppm Zn: 20.6–45.9 ppm	High yield potential, resistant to mildew and yellow rust, tolerant to high temperature during grain filling
Wheat —Zhongyou 9507*	2001	Fe: 34.9–57.8 ppm Zn: 17.7–41.1 ppm	Strong tiller capability, resistant to dry hot wind
Rice – Zhongguangxiang 1	2010	Fe: 7 ppm (polished)	Moderate resistance and yield
Maize— YR506	2015	β-carotene> 15 ppm	High yield
Sweet potato – Nanshu 010 (200730)	2010	β-carotene: 93 ppm	Low to medium starch content, high yield
Sweet potato – Jishu 08088	2014	β-carotene: 62 ppm	Medium starch content, high yield, resistant to root rot
Sweet potato - Xu083228	2017	β-carotene: 100 ppm	High yield (>32t/hectare), medium to high dry matter content (28%), good disease resistance
Sweet potato - Xu1021A	2017	Anthocyanin: 70 ppm	Fresh weight >30t/ha, good adaptation, dry matter content: 26%
Sweet potato —Yanshu 5	2001	β-carotene: 89 ppm	High yield, high drought tolerant

*Additional information needed to validate mineral levels in biofortified crops

Crop Multiplication and Dissemination

- During 2010–2011, 10 vitamin A-rich sweet potato cultivars were planted on demonstration plots in vitamin A-deficient areas (Sichuan, Chongqing, Jiangsu, Shandong, Fujian, Guangdong, and Guangxi). More than 20,000 virus-free sweet potato plants were tissue-cultured for dissemination to poor farmers.
- During 2011–2012, orange-fleshed and purple-fleshed sweet potato bases were built on about 60 hectares, and varieties were extended to a total of 2,000 hectares in Sichuan, Chongqing, Jiangsu, and Shandong.
- During 2010–2013, Zhongmai 175, with high zinc concentration, has been cumulatively cultivated on more than 500,000 hectares and became the check in the North China Winter Wheat Trial.

August 2014

- In 2013, the inbred parents of provitamin A maize YR 506 were planted in Yunxian and Linxiang districts of Yunnan province, and 1 ton of YR 506 seed was produced in 10 counties of the province.

Marketing

HarvestPlus-China has conducted extensive outreach and promotion of biofortification, as well as highlighting the magnitude of hidden hunger, its causes, consequences, and cost-effective solutions through newspapers, magazines and the internet. The book "Biofortification in China" was published by HarvestPlus-China in 2009 to inform the public about biofortification, and public awareness of the importance of micronutrients is rising.

Stakeholders

There are nine funded projects with 40 institutions, including universities, Chinese Academy of Agricultural Sciences, Chinese Academy of Sciences, and provincial centers for disease prevention and control, that focus on increasing iron, zinc, and vitamin A content in rice, maize, wheat, and sweet potato.

Potential Impact

According to a survey conducted by the National Statistics Bureau, micronutrient deficiencies in China result in a loss of 30 billion yuan (US$3.61 billion) annually, accounting for 3–4 percent of the gross domestic product. An efficacy study of OSP in rural Sichuan province showed that when school children 3–10 years of age ate 110 grams of OSP per day for 40 days, their serum retinol concentration improved, and vitamin A deficiency decreased from 17 percent to 1 percent.

Funding biofortification is a cost-effective investment in a more nourishing future. Biofortified crops will improve the overall nutritional status of 300 million Chinese suffering from hidden hunger and has the potential to lift millions out of poverty.

Delivery Challenges and Recommendations

- Coordination of research and delivery across programs can be difficult, and approaches differ by crop and region.
- More information is needed about the variation in mineral levels across environments, and iron and zinc levels require additional validation.
- Nutrition evidence is not complete for varieties developed under HarvestPlus-China. HarvestPlus-China will develop a more complete portfolio of evidence around nutrition and adoption.
- Standards development is needed to ensure stable and reliable quality and nutrition of biofortified crops and foods.
- A long-term commitment to mainstreaming biofortified crops in public policies and programs should be made.

Seed Systems and Private Seed Company Involvement in Biofortification

Marx Mbunji & Nicholas Mwansa (CIAT-HarvestPlus)

Since its beginning in 2003, HarvestPlus has cultivated strong partnerships with private sector seed companies to conduct research in developing, testing, and disseminating biofortified seed lines for future commercialization. Private sector partners are particularly important to product development and delivery in Zambia, where three seed companies are currently engaged in the delivery of orange maize, and in India, where numerous seed companies are engaged in crop development and identifying the best performing varieties of pearl millet and wheat for commercialization. As HarvestPlus prepares to commercialize zinc wheat in India, two partners, Sri Sai Seeds and SRC Bioseed, have produced seed for use in test marketing. Iron pearl millet hybrid varieties will be commercialized beginning in 2014 by two private seed companies, Nirmal Seeds and Shakti Vardhak. Nirmal Seeds also distributes the first open-pollinated iron pearl millet variety throughout the state of Maharashtra in India.

Zambia Case Study

Three vitamin A hybrids were released in Zambia in 2012. Licensing of three released hybrids and allocation of hybrids is the subject of a memorandum of understanding (MoU) between the Zambia Agriculture Research Institute (ZARI) and three respective seed companies – ZamSeed, Seed Co, and Kamano. HarvestPlus was involved as an ''interested party.'' Differences in adaptive pattern facilitated negotiations and allocation of individual hybrids to these seed companies under an exclusive commercialization modality. In consensus, Hybrid GV664A was allocated to Zamseed, GV665A to Seed Co, and GV662A to Kamano Seed Company; these companies were/are engaged in vitamin A maize performance testing and the identification of leads for submission to official registration trials. In addition, commercial contracts with HarvestPlus specify obligations and responsibilities of the parties, seed production and monitoring, marketing and distribution arrangements, and risk sharing.

Seed company partners receive breeder seed of parent lines from ZARI to initiate inbred line and F1 parent seed production, and for line maintenance. HarvestPlus facilitates the initial production and marketing of the biofortified maize seed with the various seed companies through activities such as promotion of the hybrids during field days and agriculture shows in both pre- and post-commercialization of the maize varieties. After commercial introduction, seed companies monitor the market demand for hybrids and collect information from their retail outlets and field representatives to forecast and implement the subsequent seed production.

Seed companies in general do not produce seed during the winter off-season. Off-season seed production requires irrigation, costs are much higher compared to the regular season, production can be risky due to low temperatures, and the turnaround time between harvest and seed marketing is very short. Furthermore, there is limited experience in off-season seed production. To increase the seed volume for 2012/13 season demonstrations and parental stocks, HarvestPlus contracted seed production during the 2012 winter season on a commercial farm. Seed production was below target due to irrigation management issues; lessons learned were shared with seed company partners to be applied in future off-season seed production. In 2012/13, seed company seed production focused on bulking pre-basic and some basic seed for large commercial rollout in 2014/15. In 2014, about 200 tons of seed is expected to be produced by Zamseed, while Kamano and Seed Co continue to multiply their parent lines for 2015 seed production. This seed from Zamseed is already targeted to various development agencies and farmers. Some development partners are invested in school feeding programs, while others will mobilize for commercial processing by milling companies. Any remainder of the seed shall be distributed in the normal seed retailing outlets (agrodealer network) of seed companies. HarvestPlus supports the wider distribution and promotion of the orange maize seed with the distributing seed companies.

To test the market, HarvestPlus has been engaged since 2013 with a milling company (Star Milling) to produce orange maize flour for commercial marketing as a new product line for the company. The new brand of mealie meal sells under an orange label and has been available in commercial retail chain stores like Spar and Pick'n Pay. Maize used for this purpose has been mobilized from the early adopters of small producers in the facilitation districts. The test sales have been positive in ensuring future commercial sales and promotions. With the expected collaboration with other partners promoting the commercialization of vitamin A maize, HarvestPlus expects a great future for orange maize in terms of crop production, processing, and consumption in Zambia.

Challenges

- Bulking up of parent seed is slow to meet created demand.
    - **Recommendation:** Bulking of parent seed was extended to the off-season (winter) production in order to fast track the commercial production of the released varieties. In future, seed company partners should be identified early so that parent line multiplication (pre-basic and basic seed) can begin at the same time that a candidate variety is submitted for performance testing, regardless of whether some candidates may be dropped. This will allow for adequate production to meet expected demand for the varieties at the time of release.
- Seed companies' capacity to maintain parent lines is limited.
    - **Recommendation:** Seed companies that may not have the facilities and capacity to maintain the parent lines of a licensed variety should be supported by services from the National Research System. If such capacity is wanting, services of a nearby CGIAR center should be sought to uphold the integrity (genetic purity) of the variety and the exclusivity to the variety by the seed business.
- Differentiation of vitamin A orange maize from yellow maize is needed in the markets.
    - **Recommendation:** Because not all yellow or orange maize varieties contain high levels of provitamin A (especially beta-carotene), it is important to develop innovative ways to differentiate vitamin A maize from ordinary yellow or orange maize. Strong branding with the HarvestPlus brand that has become synonymous with biofortification would be required for vitamin A maize products.

HarvestPlus leads a global effort to improve nutrition and public health by developing and deploying staple food crops that are rich in vitamins and minerals. We work with diverse partners in more than 40 countries. HarvestPlus is part of the CGIAR Research Program on Agriculture for Nutrition and Health (A4NH). CGIAR is a global agriculture research partnership for a food secure future. Its science is carried out by its 15 research centers in collaboration with hundreds of partner organizations. The HarvestPlus program is coordinated by two of these centers, the International Center for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI).

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