Biofortification: from discovery to impact

From assessment to solutions

Erick Boy
HarvestPlus / IFPRI-CIAT
e.boy@cgiar.org
Biofortification in a nutshell
The nutrition research plan
Update on results
Summary
HarvestPlus Biofortification
(Concept)

- Increases staple crop nutrient concentration without sacrificing agronomic traits (i.e. yield, pest resistance, drought resistance)
- Focus on iron, provitamin-A carotenoids, zinc
- Focus on traditional plant breeding
- Targets women and children > 2 years
- Targets the poor rural farming populations (Sub-Saharan Africa, South Asia, Latin America & Caribbean)
- Up front investment in research & development → sustainable public good
Another weapon in the armamentarium to fight micronutrient deficiencies

Supplementation

Commercial & point of use fortification

Dietary diversity

Biofortification
Linking agriculture and nutrition - a new paradigm: from farm to cell
How do we know that biofortification works?

Q#1: Does the biofortified crop contribute >30% EAR* of provitamin A, iron or zinc to target population?
- Post harvest nutrient retention studies
- Background food processing and dietary intake studies

Q#2: Are the micronutrients in the biofortified food crops bioavailable (absorbed and utilized) when consumed by the target population group(s)?
- Anti-nutrient analysis, In vitro & animal bioavailability models
- Bioavailability studies in humans

Q#3: Does consumption of biofortified foods improve micronutrient status of women and children?
- Efficacy studies
- Effectiveness studies

*EAR = estimated average requirement of a nutrient per day
In other words, nutrition research validates the initial assumptions.

Validating minimum target levels:
- Efficacy (randomized controlled trials)
  - Effectiveness trials

Flowchart:
- Development
  - Average daily food consumption
  - Nutrient retention in food
  - Bioavailability
- Evaluation

Source: Food & Nutrition Bulletin 28 (2), S271-79
## Nutrition research to date

<table>
<thead>
<tr>
<th></th>
<th>Dietary intake &amp; nutritional status</th>
<th>Nutrient retention</th>
<th>Absorption/bioavailability</th>
<th>Efficacy</th>
<th>Effective-ness</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sweet Potato</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Maize</strong></td>
<td>✓</td>
<td>✓</td>
<td>2✓</td>
<td>2✓</td>
<td>2015</td>
</tr>
<tr>
<td><strong>Cassava</strong></td>
<td>✓</td>
<td>✓</td>
<td>2✓</td>
<td>✓</td>
<td>2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2013-14*</td>
</tr>
<tr>
<td><strong>Beans</strong></td>
<td>✓</td>
<td>✓</td>
<td>3✓</td>
<td>2✓</td>
<td>2014?</td>
</tr>
<tr>
<td><strong>Pearl millet</strong></td>
<td>✓</td>
<td>✓</td>
<td>2✓</td>
<td>✓</td>
<td>2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2013-14*</td>
</tr>
<tr>
<td><strong>Rice</strong></td>
<td>✓</td>
<td>✓</td>
<td>2013</td>
<td>2013-14*</td>
<td>2016</td>
</tr>
<tr>
<td><strong>Wheat</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>2013-14 (2)*</td>
<td>2016</td>
</tr>
</tbody>
</table>

**Complete:** ✓  **Ongoing:** ➔  **Period of implementation:** *

* Period of implementation
Q#1 Can breeding increase nutrient levels enough to improve human nutrition? Can the nutritional target level be reached in crops.
Nature gives: genetic variation, baseline & target levels
(Fe = iron; Zn = zinc; pVAC = provitamin-A carotenoids)
Breeding target should provide % of the estimated average requirement (EAR) that produces measurable changes in nutritional status.
Original plant breeding targets for vitamin A (Vit. A), iron (Fe), and zinc (Zn) for a non pregnant, non lactating woman

<table>
<thead>
<tr>
<th>Nutrient &amp; crop</th>
<th>Food consumed (g/day)</th>
<th>“Additional nutrient concentration (μg/g)”</th>
<th>Nutrient Retained after storage &amp; processing (%)</th>
<th>Absorbed proportion (%)</th>
<th>Nutrient absorbed (μg/day)</th>
<th>Daily requirement for non pregnant, non lactating woman (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize &amp; cassava</td>
<td>400</td>
<td>15.0</td>
<td>50</td>
<td>8.0</td>
<td>240</td>
<td>Vit. A = 500</td>
</tr>
<tr>
<td>Beans</td>
<td>200</td>
<td>44.0</td>
<td>90</td>
<td>5.0</td>
<td>396</td>
<td>Iron = 1460</td>
</tr>
<tr>
<td>Rice &amp; wheat</td>
<td>400</td>
<td>8.0</td>
<td>90</td>
<td>25.0</td>
<td>720</td>
<td>Zinc = 1860</td>
</tr>
</tbody>
</table>

Nutrient absorbed
Nutrient required = % EAR

= 48% Vit.A

= 27% Fe

= 39% Zn
Q#2: Are the micronutrients in the biofortified food crops bioavailable (absorbed and utilized) when consumed by the target population group(s)?
Provitamin-A Maize: Zambia studies

Maize consumption & Vit. A status
(2 districts 2009)
- 2010: vit.A deficiency in children: 48%;
  62% infected (16% malaria)
- Average maize intake: Women: 287g/day
  Children: 128 g/day

Retention (2009-10)
- 75% after cooking
- ~50% after storage
  4-6 m→37.5% overall retention

Bioavailability (2009, 2011)
- 6.5 beta carotenes per 1 retinol (16% availability)
  (Source: Li et al 2010)
- 3 beta carotenes per 1 retinol (33%) (source: Muzhingi et al 2011)

Efficacy (2012-14)
- Johns Hopkins Univ.: population serum retinol, immune response, dark adaptation (2013);
- UCD: breast milk retinol (2013-14)
Given these data, can biofortified maize still contribute >50% vitamin A requirement of women in rural Zambia? YES

- Full nutrient target concentration in biofortified crop (raw food): 15 μg/g
- Nutrient retained after: storage 50%, & cooking 75% → 37.5%
- Amount of maize consumed: 287 g/day by adult woman
- % nutrient bioavailable → 17% (6:1)
- Contribution to estimated average requirement of non pregnant, non lactating women: 54%
Provitamin-A Cassava: Nigeria

Food consumption & VA status Survey of Akwa Ibom State (West) (2011)

- Vit. A deficiency in 32% of children & 3% of women
- Women consume ~940g/day; Children 3-5 years consume 358g/day; (fresh weight cassava)

Retention, 2009-10

- 2011 review: 70% if boiled
- 2012: retention in freshly made gari (40%)*

Bioavailability: 2009 & 2011

- 2.8 beta carotenes per 1 retinol (33% availability)
  (Source: Liu W. 2009)
- 4.1:1 & 4.7:1 (w/oil);
  (Source: La Frano et al, 2012)

Efficacy

- 2012: Improved serum retinol (p=0.04) in Kenya (Wageningen Agricultural University)
- 2013-14 Nigeria Request for proposals stage
High vitamin A equivalence of biofortified cassava with or without oil

<table>
<thead>
<tr>
<th></th>
<th>Biofortified cassava with oil <em>by weight</em></th>
<th>Biofortified cassava without oil <em>by weight</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± standard deviation</td>
<td>4.14 ± 2.23</td>
<td>4.68 ± 2.94</td>
</tr>
</tbody>
</table>

= molecules of β-carotene in biofortified cassava (nmol) 
= molecules of retinol formed (nmol)

*Source: La Frano MR, et al. AJCN, 2012; doi:10.1017/S0007114512005004
Retinol Equivalency of provitamin A rich foods: summary of human studies

Bioequivalence Factor: number of beta carotene molecules needed to produce one molecule of retinol

- Golden rice
- Orange maize
- Cassava
- Orange sweet potato
- Orange/yellow fruit
- Orange fruit
- Carrots
- Indian spinach
- Spinach
- Green leafy/carrots
- Dark green/yellow

“12” was the bioequivalence factor assumed initially (lower is better)
Provitamin A crops research summary: sweet potato, maize, cassava

- Orange fleshe sweet potato (OFSP): residual impact of 2 year intervention on OFSP and vit. A intake 2 years post effectiveness trial in Mozambique
- Orange maize: 3 comprehensive efficacy trials in Zambia in 2013-14 (Johns Hopkins University, University Wisconsin, University of California)
- Yellow cassava:
  - Data analysis of efficacy trial with boiled cassava for school children in rural Kenya (Wageningen Agriculture University)
  - Efficacy trials in Nigeria: 2013-2014
Minerals: what about iron-biofortified crops? beans and pearl millet

Q#3: Does consumption of biofortified foods improve micronutrient status of women and children?
Beans efficacy trial among school children in Oaxaca, México (Instituto Nacional de Salud Pública and Cornell University, 2009-10)
Comparison of iron concentration distributions in control and intervention beans: Mexico
(μg of iron per gram of dry beans)

Bean variety NG8025             Bean Variety MIB465

Difference = 40 μg/g
Higher bean consumption associated with better iron status (high iron beans efficacy trial - Oaxaca, Mexico)

The average additional iron intake from ~64 g biofortified beans/day (mg/d) = 3.26 mg (76% higher than controls; 12% of the daily iron requirement)

Difference in iron status (improved tissue iron concentration as indicated by transferrin receptors, sTfR) = -0.410 sTfR, mg/L (p=0.02)
The change in transferrin receptors (sTfR) increased with degree of deficiency at baseline.

The greatest effect of consuming high iron beans was in the most iron deficient children.

- Baseline sTfR
  - Sufficient: 4.38 mg/L
  - Deficient: 4.89 mg/L

- Change in sTfR:
  - Sufficient: 0.16 mg/L
  - Deficient: 0.50 mg/L

- Percentiles:
  - 25th: 4.38 mg/L
  - 50th: 4.89 mg/L
  - 75th: 5.49 mg/L
Lowering phytate: fractional iron absorption and iron absorbed per test meal with low phytic acid (*lpa*) beans$^{1,2}$

<table>
<thead>
<tr>
<th>Bean variety in the meal</th>
<th>Fractional iron absorption$^3$</th>
<th>Total iron absorbed per meal$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>Parent- <em>lpa</em> brown</strong></td>
<td>20</td>
<td>a,c3.84 (1.76; 8.38)</td>
</tr>
<tr>
<td><strong>lpa brown</strong></td>
<td>20</td>
<td>b6.14 (2.57; 14.65)</td>
</tr>
<tr>
<td><strong>Parent - <em>lpa</em> white</strong></td>
<td>20</td>
<td>a2.68 (1.26; 5.69)</td>
</tr>
<tr>
<td><strong>lpa white</strong></td>
<td>20</td>
<td>b,c3.99 (1.83; 8.71)</td>
</tr>
</tbody>
</table>

$^1$All meals contained 2 mg Fe$^{57}$ or 2 mg Fe$^{58}$

$^2$Values in a column with superscripts without common letter differ, P< 0.05 (repeated measure ANOVA, Bonferroni adjustment)

$^3$Values are geometric means; 95% CIs in parentheses. **Source:** J Nutr 2013 Jun 19. [Epub ahead of print as as doi: 10.3945/jn.113.175067]
Iron Biofortified Pearl Millet Improves Iron Status in Indian School Children - *Results of a Feeding Trial*

- Jere D. Haas¹, Julia L. Finkelstein¹, Shobha A Udipi²,
- Padmini Ghugre², Saurabh Mehta¹

¹ Division of Nutritional Sciences, Cornell University, Ithaca, NY, ²S.N.D.T Women's University, Mumbai, India

Paper presented at the annual meeting of the American Society for Nutrition
Boston MA
April 22, 2013

Funding from *HarvestPlus™*
Children consumed on average 1.13 bhakri per meal or 232 g pearl millet flour/day resulted in:

- 19.4 mg/d iron from biofortified bhakri
- 5.3 mg/d iron from the control bhakri

(estimated average requirement for age group = 6-8 mg/d)
Iron Deficiency and Resolution of Iron Deficiency

The group consuming biofortified pearl millet reduced the prevalence of iron deficiency (Ferritin <15mg/L) from 47% to 23% over the 6 months of the study.

The control pearl millet also reduced iron deficiency, from 39% to 27%.

40% of iron deficient control pearl millet resolved their deficiency, while 65% of the iron deficient biofortified subjects resolved their deficiency after 6 months.

The biofortified pearl millet was 1.64 times more effective in resolving iron deficiency (Relative Risk = 1.64, CI 1.08,2.49; p=0.02)
Comments and conclusions

• Biofortified pearl millet is efficacious in improving iron status
• The large quantity of pearl millet consumed by children in this study resulted in measurable results in 3-4 months
• Non-fortified control pearl millet consumed at 200-300 g/d also resulted in significant improvements in iron status, but required more time.
• Consumption of biofortified pearl millet resolved 64% more iron deficiency as lower dose controls
Iron Crops Research

- Longer term iron absorption trials:
  - Iowa State University: adaptation to inhibitory effect of phytate on iron absorption (ongoing)
  - Beans efficacy trial in Rwanda: January-June 2013. Analysis stage.
- Mexico beans efficacy trial showing improvement in iron status of school children (to be published)
- Breeding for lower phytate contents in beans: low phytate lines of white and red beans will be tested for iron absorption in Rwanda in 2013.
Zinc crops: wheat, rice, and pearl millet
Zinc absorbed from 87 g of biofortified pearl millet is more than adequate to meet requirement of children 2-4 years of age (Belgaum, India)

<table>
<thead>
<tr>
<th>Pearl millet group</th>
<th>Age (months)</th>
<th>Fractional absorption of Zn</th>
<th>Total absorbed Zn, mg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. High iron &amp; zinc</td>
<td>28±4</td>
<td>0.171 ± 0.08</td>
<td>0.95 ± 0.47</td>
</tr>
<tr>
<td>B. Low iron &amp; zinc</td>
<td>29 ± 3</td>
<td>0.202 ± 0.043</td>
<td>0.67 ± 0.24</td>
</tr>
<tr>
<td>p -value</td>
<td>0.32</td>
<td>0.15</td>
<td>0.03</td>
</tr>
</tbody>
</table>

A: 84 ug Zn/g millet and 7.5 mg phytate/g millet
B: 43 ug Zn/g millet and 10.3 mg phytate/g millet

Wheat: more zinc absorbed (mg/day) from biofortified wheat than from control flours (80% and 95% extracted)

<table>
<thead>
<tr>
<th>Flours (% wheat extraction)</th>
<th>Wheat</th>
<th>Total zinc intake (mg)</th>
<th>Fractional zinc absorbed (%)</th>
<th>Total zinc absorbed (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>Control</td>
<td>3.9±0.1</td>
<td>38±1.0</td>
<td>1.5±0.5(^a)</td>
</tr>
<tr>
<td>80%</td>
<td>High zinc</td>
<td>6.6±0.1</td>
<td>31±1.0</td>
<td>2.0±0.5(^b)</td>
</tr>
<tr>
<td>95%</td>
<td>Control</td>
<td>7.9±0.2</td>
<td>20±5.0</td>
<td>1.6±0.4(^c)</td>
</tr>
<tr>
<td>95%</td>
<td>High zinc</td>
<td>13.6±0.4</td>
<td>15±5.0</td>
<td>2.1±0.7(^d)</td>
</tr>
</tbody>
</table>

HOWEVER, zinc requirements used for calculating targets underestimate needs

**Gap between Institute of Medicine (IOM) – IZiNCG requirements**

- IZiNCG
- International Zinc Nutrition Consultative
- Institute of Medicine (www.iom.edu)

Current or “real” gap since revision of International Zinc Nutrition Consultative

- Data correction
- Elimination of n-weighted regression
- Body Weight
- MENSES

Gap:

- IZiNCG: 1.86
- International Zinc Nutrition Consultative: 2.32
- Body Weight: 2.67
- IOM: 3.2
- Current or “real” gap: 2.89
"The estimates of zinc physiological requirements by International Zinc Nutrition Consultative Group (IZiNCG) in 2004 were conspicuously low in comparison with those estimated by the Institute of Medicine (IOM) in 2001."


Higher physiological zinc requirements for women: from 1.86 to ~2.5-3.0 mg/day for women
• Adequate zinc absorption from pearl millet and wheat
• Two zinc-wheat efficacy trials in India: women and children under 2 years in New Delhi slums & in rural Haryana. (Preparatory stage)
• One zinc-and-iron pearl millet efficacy trial in India: children under 2 years – call for proposals concluded. Funding granted.
• Zinc targets in rice & wheat being revised up.
Research findings and gaps

• **Provitamin A (again the strongest story):**
  – Efficacious and effective orange flesched sweet potato.
  – Highly bioavailable carotenoids in cassava and maize
  – Considerable degradation of carotenoids in cereals and flours during storage

• **Iron:**
  – Highly efficacious pearl millet
  – Potentially efficacious common beans (lower phytate required)

• **Zinc:**
  – Increase in breeding target is likely due to adjustment for revised physiological requirements
# Nutrition research to date

<table>
<thead>
<tr>
<th>Date</th>
<th>Dietary intake &amp; nutritional status</th>
<th>Nutrient Retention</th>
<th>Absorption/bioavailability</th>
<th>Efficacy</th>
<th>Effective-ness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Ongoing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sweet potato**

- ✓
- ✓
- ✓
- ✓
- ✓

**Maize**

- ✓
- ✓
- 2✓
- 2✓
- 2015

**Cassava**

- ✓
- ✓
- 2✓
- ✓
- 2013-14*
- 2015

**Beans**

- ✓
- ✓
- 3✓
- 2✓
- 2014?

**Pearl millet**

- ✓
- ✓
- 2✓
- ✓
- 2013-14*
- 2015

**Rice**

- ✓
- ✓
- 2013
- 2013-14*
- 2016

**Wheat**

- ✓
- ✓
- ✓
- 2013-14 (2)*
- 2016

* Period of implementation
THANK YOU