# Biofortification:

## Breeding for Micronutrient Enrichment
## &
## Prospects in Sorghum

**Hariprasanna K.**

**hari@sorghum.res.in**

ICAR-Directorate of Sorghum Research
Rajendranagar, Hyderabad - 500 030
*www.sorghum.res.in*

# Agricultural Systems = Food


- Mostly focus on increased productivity & profitability for farmers and agricultural industries
- Never explicitly designed to promote human health

**Health comes from the pharmacy!**

# Human Requirement

## The 49 known essential nutrients for sustaining human life

| | |
|---|---|
| Water and energy | Water, Carbohydrates |
| Protein (amino acids) | Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine |
| Lipids-fat (fatty acids) | Linoleic acid, Linolenic acid |
| Macro-elements | Na, K, Ca, Mg, S, P, Cl |
| Micro-elements | Fe, Zn, Cu, Mn, I, F, B, Se, Mo, Ni, Cr, Si, As, Sn, Co |
| Vitamins | A, D, E, K, C, $B_1$, $B_2$, $B_3$, Niacin, $B_6$, $B_9$, Biotin, $B_{12}$ |


**Malnutrition** both under & over-nutrition leading to obesity - Dysfunctional food systems that cannot supply all the nutrients and health-promoting factors

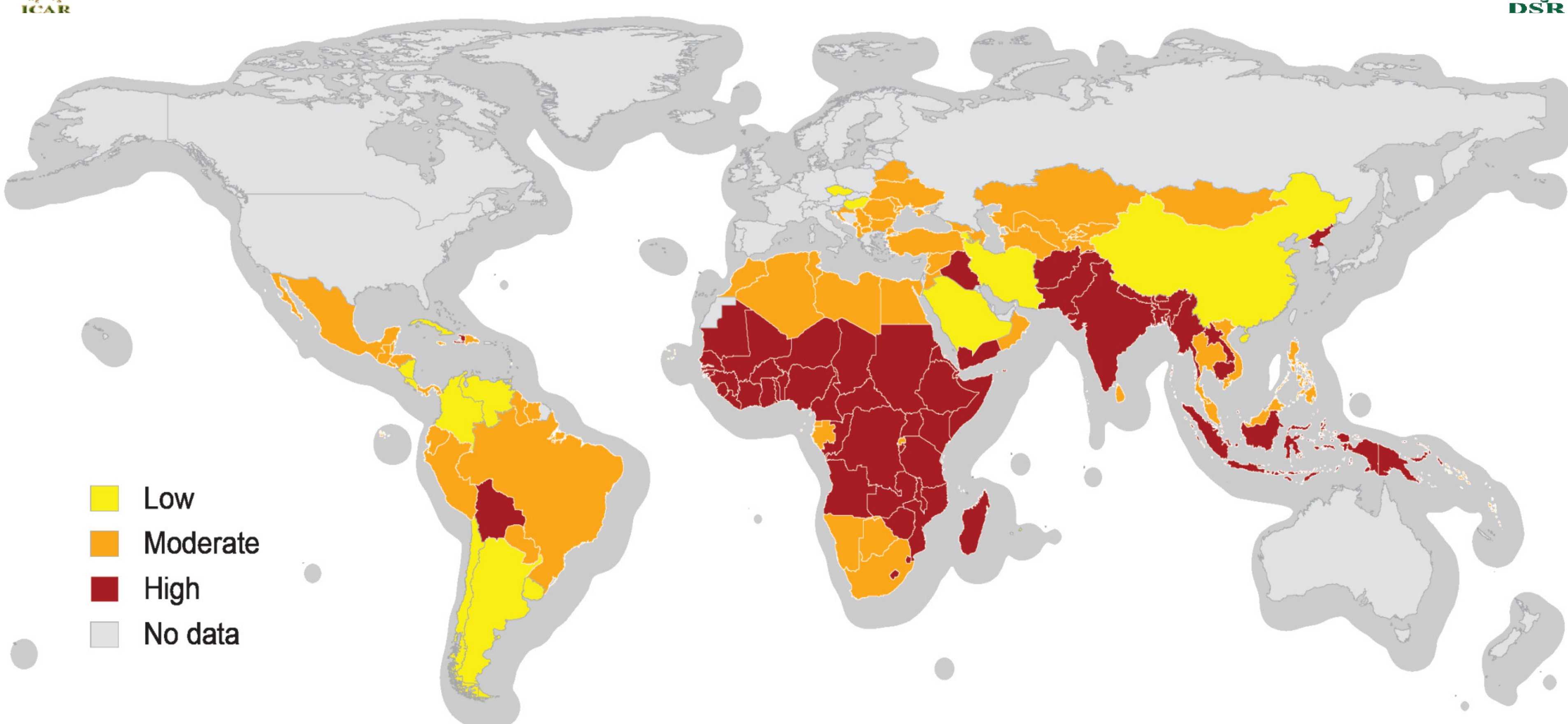
Malnutrition caused by deficiencies of vitamins and minerals - '**Hidden hunger**'; affected people do not show the physical symptoms usually associated with hunger and malnutrition

# Micronutrient Malnutrition (MNM)

- Global serious food related health problem
- Afflicts more than ½ of the developing world's population or > 2 billion people, especially the women and preschool children
- Nearly 2/3rd of all deaths of children are associated with nutritional deficiencies, many from micronutrients
- UN General Assembly adopted MDGs (2001) - Fighting MNM is an integral component

- **Poor people living in the arid and SAT regions suffer most from micronutrient deficiencies as they cannot afford a variety of food items in their diet**

# Micronutrient Malnutrition

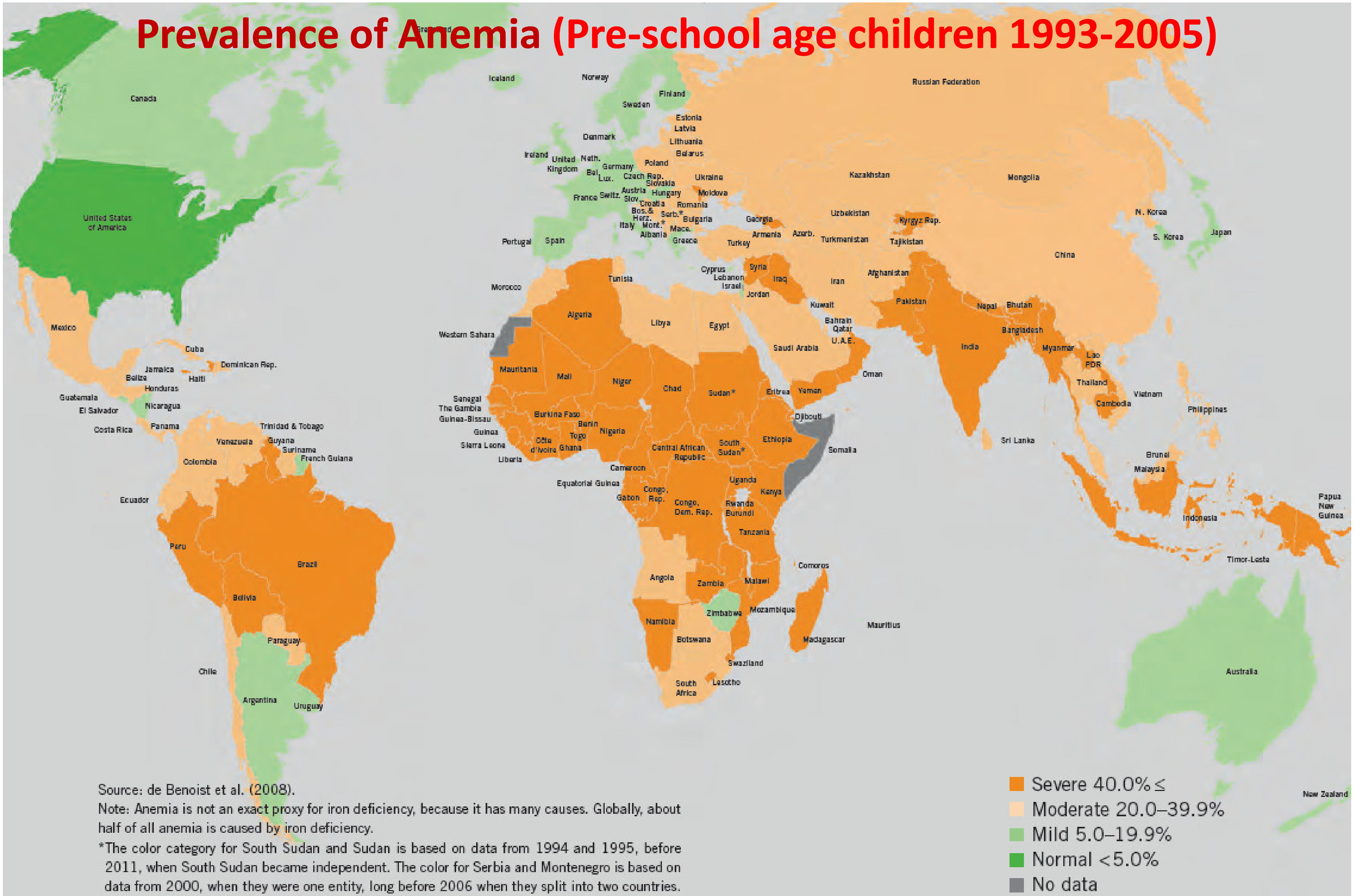



**Global Prevalence of Micronutrient Deficiencies**

- **Vit. A: Vision, immune response, reproduction, embryonic development, etc.**
- **Iron: Anaemia, Cognitive development, resistance to infection, work capacity, productivity, etc.**
- **Zinc: Impaired growth, immune disfunction, mortality, adverse pregnancy outcomes, abnormal neuro-behavioural developments**

# Micronutrient Malnutrition

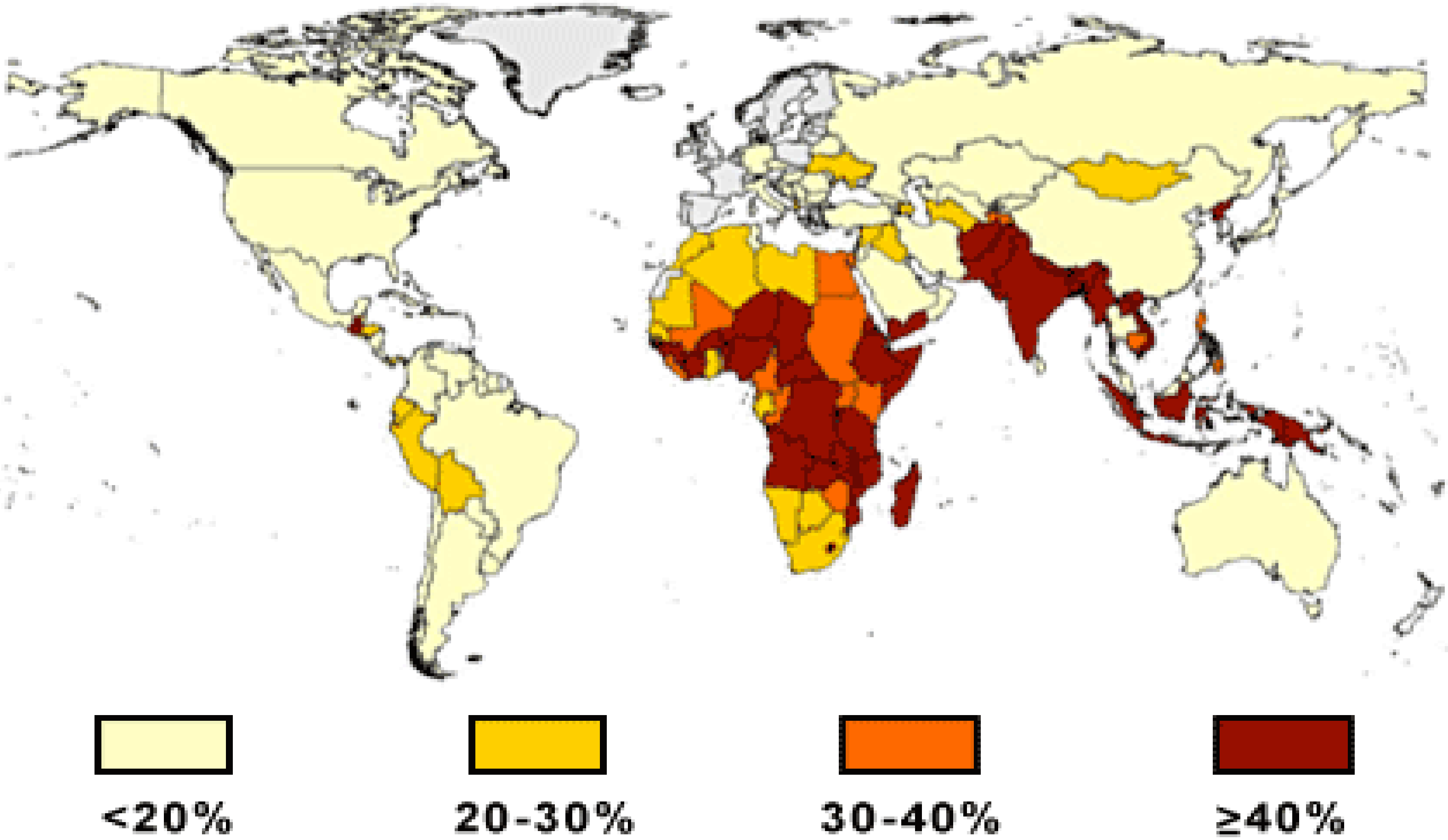
## Prevalence of Anemia (Pre-school age children 1993-2005)




Source: de Benoist et al. (2008).
Note: Anemia is not an exact proxy for iron deficiency, because it has many causes. Globally, about half of all anemia is caused by iron deficiency.
*The color category for South Sudan and Sudan is based on data from 1994 and 1995, before 2011, when South Sudan became independent. The color for Serbia and Montenegro is based on data from 2000, when they were one entity, long before 2006 when they split into two countries.

# Micronutrient Malnutrition

## Prevalence of nutritional stunting (children under 5 years of age) (proxy to estimate risk of zinc deficiency)



# Micronutrient Malnutrition

**Baby**
- Low birth weight
- Higher mortality rate
- Impaired mental devpt.

**Child**
- Stunting
- Reduced mental capacity
- Frequent infections
- Reduced learning capacity
- Higher mortality rate

**Adolescent**
- Stunting
- Reduced mental capacity
- Fatigue
- Increased vulnerability to infection

**Pregnant women**
- Increased mortality
- Increased perinatal complications

**Adult**
- Reduced productivity
- Poor socioeconomic status
- Malnutrition
- Increased risk of chronic disease

**Elderly**
- Increased morbidity (including osteoporosis & mental impairment)
- Higher mortality rate

# What is Biofortification?

**The development of micronutrient-dense staple crop varieties using the best traditional breeding practices and modern biotechnology**

- **Fortification** - the addition of an ingredient to food to increase the concentration of a particular element (Iodised salt, Vit. A and D in margarine)
- **Supplementation** - the addition of an element to the diet to make up for an insufficiency (Vitamin capsules)

**"Health comes from the farm, not the pharmacy"**

# Why Biofortification?

- Fortification and supplementation are shorter term public health interventions; most appropriate for acute cases of micronutrient deficiency
- Require infrastructure, sophisticated processing technology, product control, purchasing power, access to markets and health care system for their success
- Not available to people living in remote areas
- **Agronomic practices** to increase micronutrient content of cereals – Soil/foliar fertilization – not feasible, costly, specific agrl. practices, etc.

# Biofortification

**Advantages**

A powerful intervention tool

- Implicitly targets low income house-holds: capitalizes regular daily intake of staple
- Reach the poor in rural areas with poor access to markets or health care systems
- One-time investment – seeds that fortify themselves: shared
- Low recurrent costs – cost of seed production and deployment: Cost effective
- Sustainable in the longer term, varieties will continue to be grown and consumed year after year
- Relies on the plant's biosynthetic (Vit.) or physiological (mineral) capacity: no effect of policy change or weak funding

# Biofortification

**Criteria**

- Crop productivity must be maintained /enhanced to guarantee farmer acceptance (high yielding)
- Micronutrient enrichment levels must have significant impact on human health (effective)
- Enriched levels must be relatively stable (stability)
- Bioavailability in enriched lines must be tested in humans to ensure that they improve the micronutrient status of people preparing and consuming them (efficacious)
- Consumer acceptance has to be tested (taste and cooking quality)

# Biofortification

**Steps**

- Identification of genetic variability within the range that can influence human nutrition
- Introgressing this variation into high yielding, stress tolerant genotypes possessing acceptable end-use quality attributes
- Testing the stability of micronutrient accumulation across the target environment
- Large scale deployment of seed of improved cultivars to farmers

**Biofortification requires a multidisciplinary research approach**

- Direct linkages between agricultural researchers and various specialists like nutritionists, public health officials, sociologists, political scientists, food technologists and economists

# HarvestPlus

HarvestPlus is a global alliance of institutions and scientists seeking to improve human nutrition by breeding new varieties of staple food crops consumed by the poor that have higher levels of micronutrients, through a process called biofortification

It is an initiative of the Consultative Group on International Agricultural Research (CGIAR). It is coordinated by the International Centre for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI).

**85 % of HarvestPlus resources for conventional breeding**

**Grand Challenges in Global Health initiative of the Bill & Melinda Gates Foundation is funding biofortification projects on banana, cassava and sorghum for Africa.**

# HarvestPlus's Strategy

**Discovery**
Identify target populations & set nutrient targets
Validate nutrient targets
Discover & screen crop genes

↓

**Development**
Improve & evaluate crops
Test nutritional efficacy of crops
Study farmer adoption & consumer acceptance

↓

**Delivery**
Release & disseminate crops in target countries
Promote consumption of crops

↓

**Measure crop adoption & improvements in nutritional status**

| Phase I (2004-07) Crops | Phase II (2009-13) Crops |
|---|---|
| Rice | Peanut |
| Wheat | Lentil |
| Maize | Cowpea |
| Beans | Pigeon pea |
| Cassava | Sorghum |
| Sweet potato | Pearl millet |
| | Barley |
| | Banana/ Plantain |
| | Potato |
| | Yams |

**Discovery research**

Have already completed an exploration of the germplasm and initial studies of the genetics and G × E interactions

**Development** of specific biofortified products in target countries with specific nutrition objectives

**Bill and Melinda Gates Foundation funded Grand Challenges 9 is developing transgenic crops**

# HarvestPlus's Strategy

## HarvestPlus III (2014-2018)

- **Demonstrate the viability of biofortification as a global solution**
- **Scale up delivery in target countries and expand delivery to new countries**
- **Strengthen the pipeline of biofortified varieties**
- **Research, communicate and advocate strategically**

| Crop | Nutrient | Target country |
|---|---|---|
| Bean | Iron (Zinc) | Rwanda, DR Congo , Brazil |
| Cassava | Provit. A | DR Congo, Nigeria, Brazil |
| | Provit. A, Iron* | Nigeria, Kenya |
| Maize | Provit. A | Zambia , Nigeria, Brazil, China, India |
| Rice | Zinc (Iron) | Bangladesh, India, Brazil |
| | Provit. A* | Philippines, Bangladesh, Indonesia, India |
| | Iron* | Bangladesh, India, China |
| Sweet potato | Provit. A | Uganda , Mozambique, Brazil, China |
| Wheat | Zinc (Iron) | India, Pakistan, China, Brazil |
| Banana/ Plantain | Provit. A | Nigeria, Cameroon, Burundi, DR Congo |
| | Provit. A, Iron* | Uganda |
| Cowpea | Iron, Zinc | India , Brazil |
| Irish potato | Iron | Rwanda, Ethiopia |
| Lentil | Iron, Zinc | Nepal, Bangladesh, Ethiopia, India, Syria |
| Pearl millet | Iron (Zinc) | India |
| Pumpkin | Provit. A | Brazil |
| Sorghum | Zinc, Iron | India |
| | Provit. A* | Kenya, Burkina Faso, Nigeria |

# β Carotene Enrichment


Orange-flesh Sweet Potato

Vit.A Maize for Nigeria & Zambia

Phytoene synthase (CrtB), Phytoene desaturase (CrtI), Lycopene beta-cyclase (CrtY) from *Erwinia herbicola* under tuberspecific/ constitutive promoter control

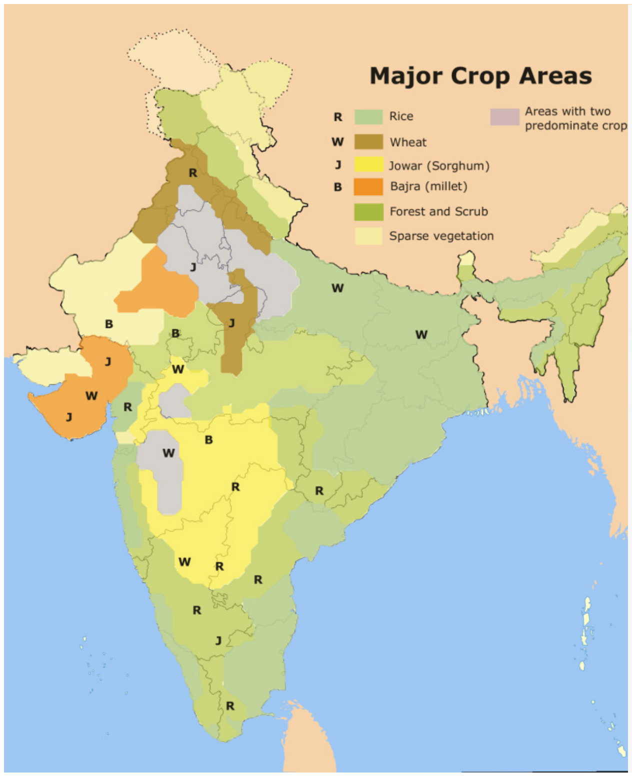
# Indian Scenario

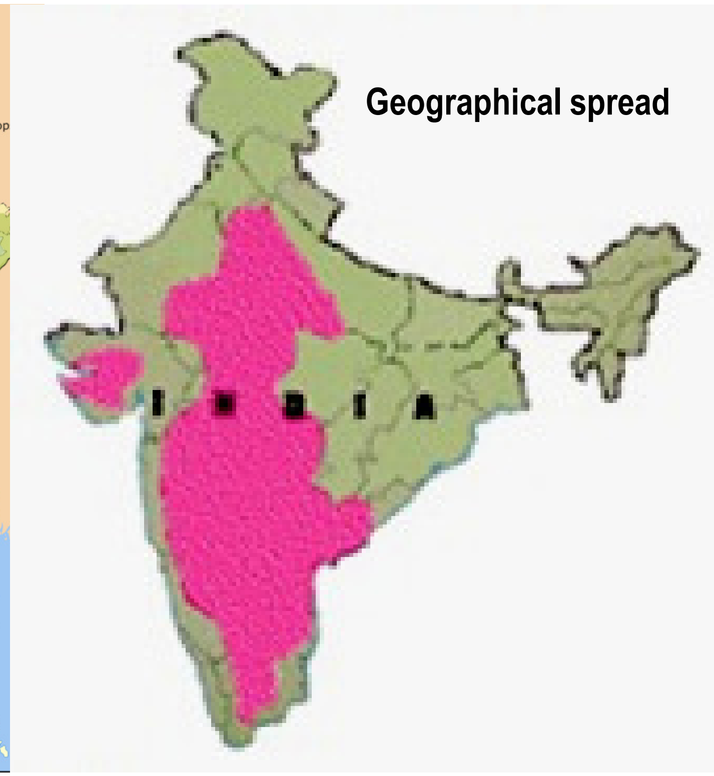
How severe is Hidden hunger?

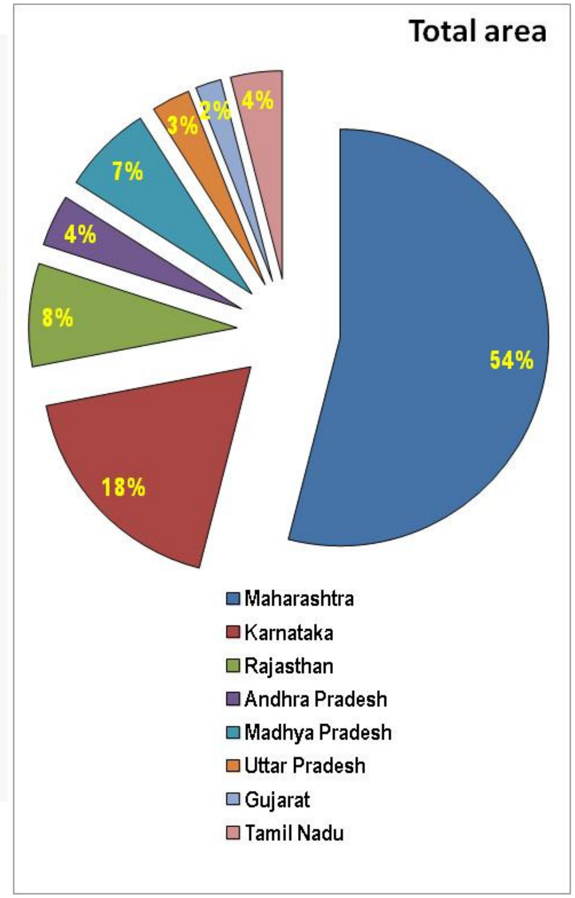
- Intake of micronutrients in daily diet is < 50% RDA in over 70% of Indian population
- Alarmingly high deficit among children, adolescents, and pregnant and lactating women
- About 57% of pre-schoolers and their mothers have subclinical VAD
- Iron deficiency anaemia (IDA) is the most serious; 62% of pre-school children are deficient in vitamin A, leading to an annual 3.3 lakh child deaths; and 58.7% of pregnant women, 63.2% lactating mothers and 69.5% of pre-school children are anaemic
- The prevalence of Zn deficiency has not been adequately investigated, partly due to lack of suitable biomarkers
- 2004 - DBT initiated the India Biofortification Programme - rice, wheat and maize biofortifed with Fe, Zn and provit. A.
- XI Plan – DBT funded biofortification of groundnut and pigeon pea for alleviating vitamin A; Sorghum biofortification for high grain Fe and Zn content
- High-iron pearl millet variety ICTP 8203Fe developed by ICRISAT was released as '*Dhanshakti*' in Maharashtra during April 2013

# Prospects of Sorghum Biofortification

# Prospects of Sorghum Biofortification

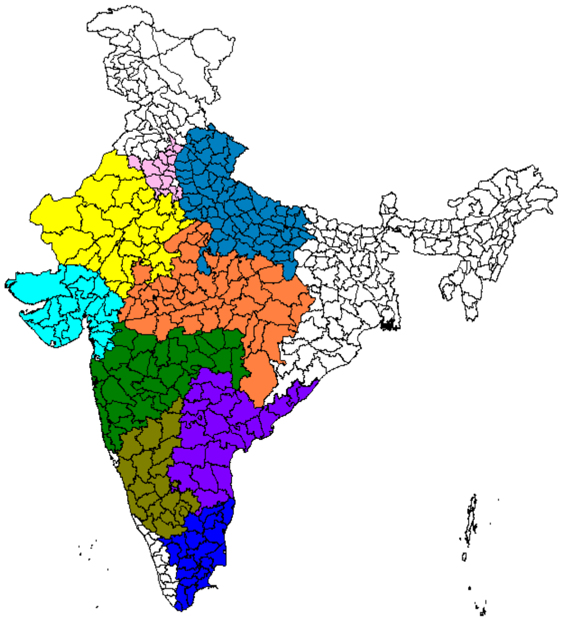


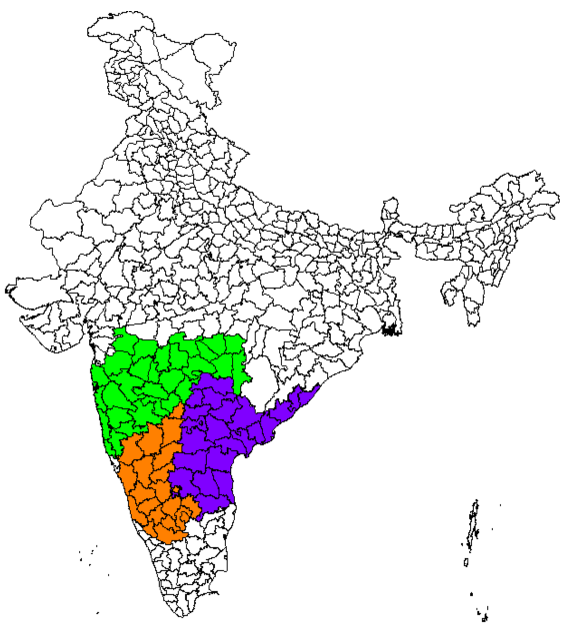




- Sorghum is the fourth most important cereal consumed
- Mostly consumed in the regions in which it is cultivated
- Maharashtra (47%), Karnataka (20%) and AP (9%) – major growing states; Staple of central and western regions of Maharashtra and the northern regions of Karnataka and Telangana

# Prospects of Sorghum Biofortification





- Inland regions of Central, Eastern and Western Maharashtra and Northern Karnataka - per capita consumption in rural areas 31.8-54.2 kg/y, urban areas 9.9-34.0 kg/y
- Accounts for about 35% of the total intake of calories, protein, Fe and Zn in the dominant consumption regions
- Cheap source of energy, protein, Fe and Zn next only to bajra among all cereals and pulses; >50% of the Fe and Zn requirements in low income group
- Sorghum biofortification will help in alleviating micronutrient malnutrition in low-income rural households in major consuming regions

# Nutritional Composition (per 100 g)

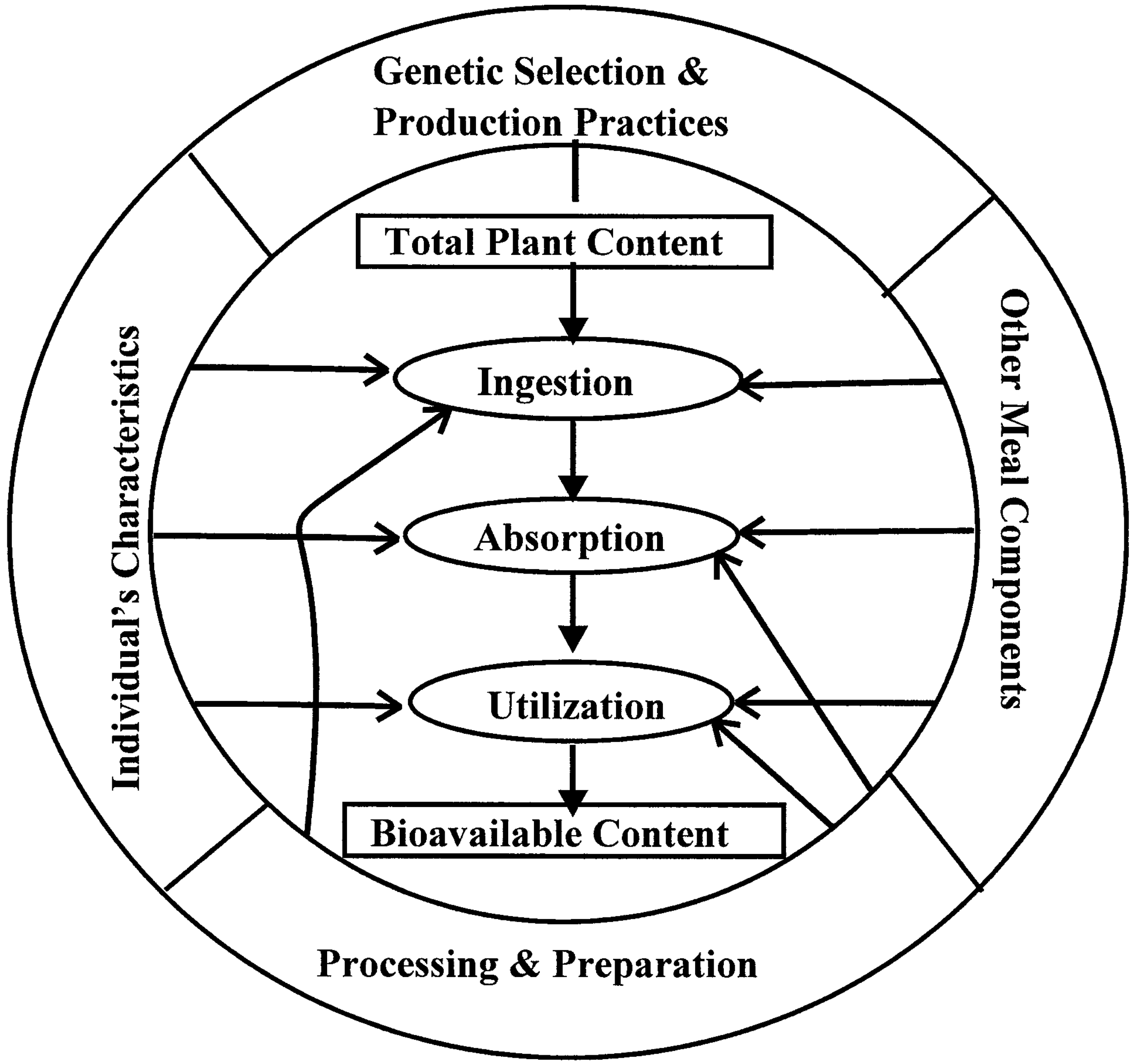
| Food grain | CHO (g) | Protein (g) | Fat (g) | Energy (KCal) | Crude fibre (g) | Minerals (g) | Ca (mg) | P (mg) |
|---|---|---|---|---|---|---|---|---|
| Sorghum | 72.6 | 10.4 | 1.9 | 349 | 1.6 | 1.6 | 25 | 222 |
| Maize | 66.2 | 11.1 | 3.6 | 342 | 2.7 | 1.5 | 10 | 348 |
| Wheat (whole) | 71.2 | 11.8 | 1.5 | 346 | 1.2 | 1.5 | 41 | 306 |
| Rice (raw, milled) | 78.2 | 6.8 | 0.5 | 345 | 0.2 | 0.6 | 16 | 160 |

(NIN, Hyderabad)

| Food grain | Fe (mg) | Zn (mg) | Fe bioaccessibility (%) | Zn bioaccessibility (%) |
|---|---|---|---|---|
| Rice | 1.32 | 1.08 | 8.05 | 21.4 |
| Maize | 3.21 | 1.48 | 7.83 | 7.82 |
| Wheat | 3.89 | 1.62 | 5.06 | 8.93 |
| Sorghum | 4.51 | 2.24 | 4.13 | 5.51 |

(Hemalatha et al., 2007. Food Chemistry 102:1328–1336)

# Bioavailability

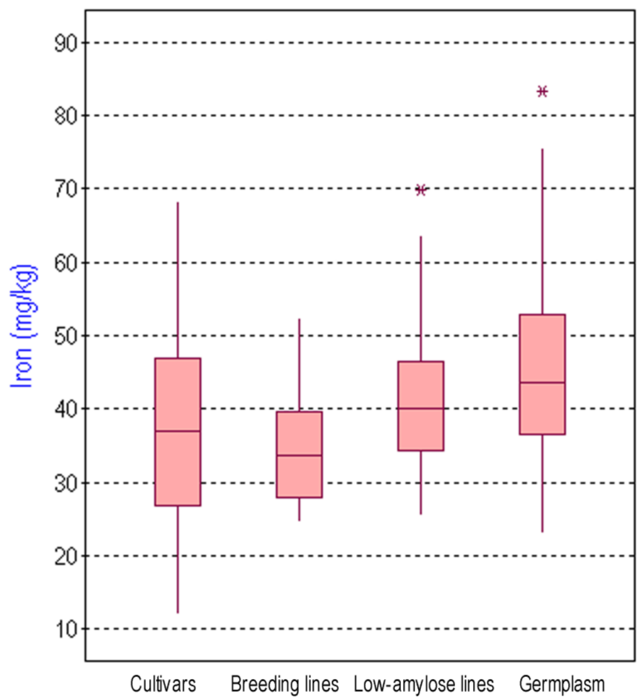


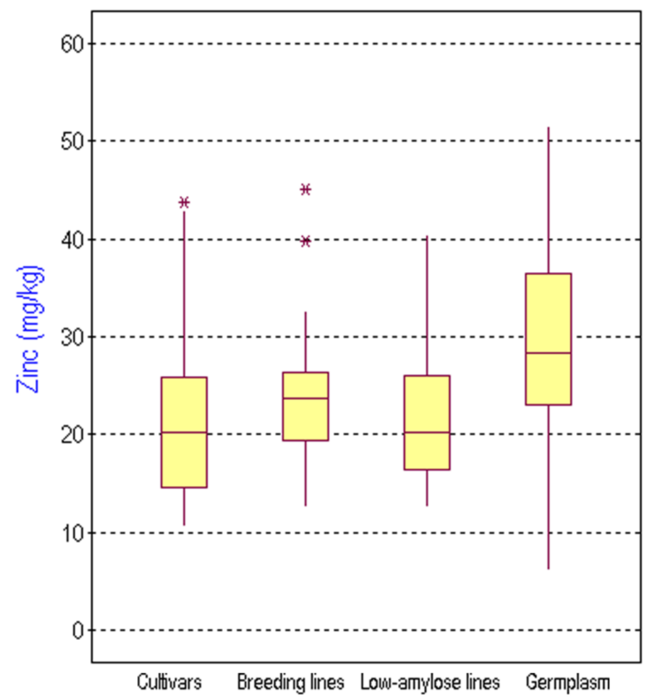

**The complexities of bioavailability in human nutriture (Graham et al., 2001).**

# Genetic Variability

## - A prerequisite for plant breeding

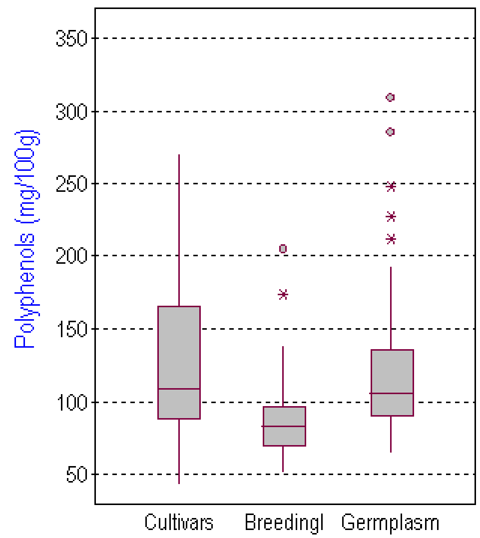
| Fe (mg/kg) | Zn (mg/kg) | Corr. Fe-Zn | Sorghum material |
|---|---|---|---|
| 12-83 | 6-51 | 0.50* | Parental lines, cultivars, advanced breeding lines & germplasm accn. (192) |
| 26-70 | 13-40 | 0.18 | Yellow sorghum & elite lines (30) |

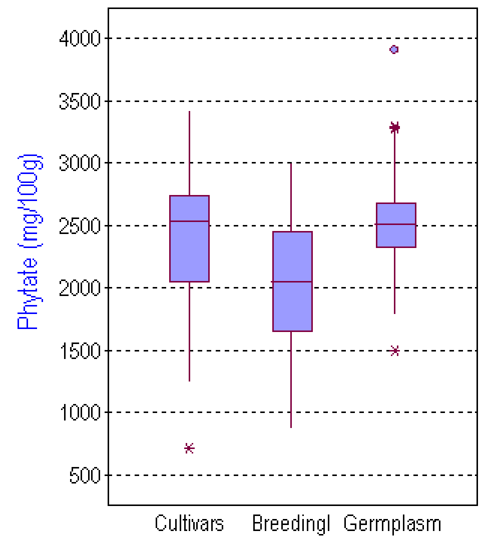


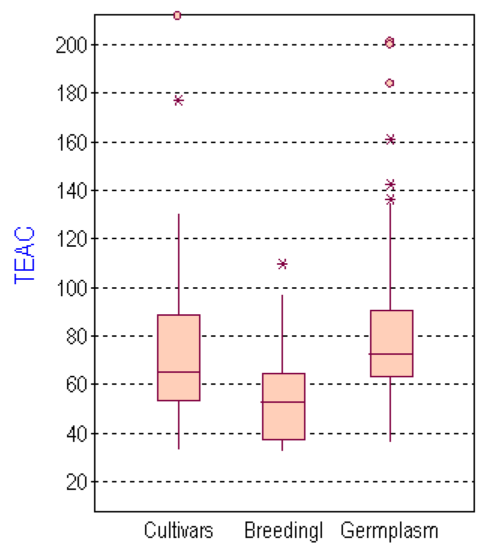



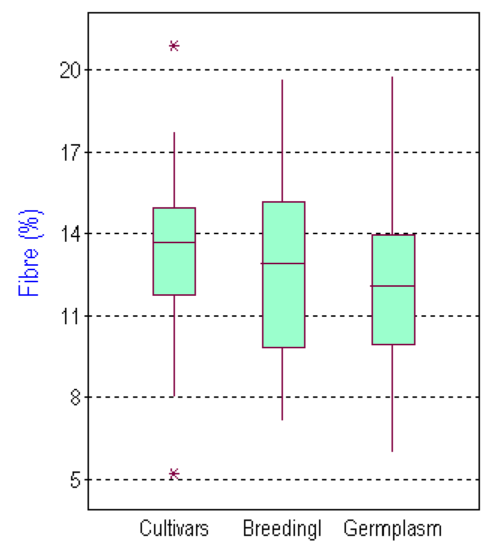

(Hariprasanna et al. 2014)

# Variability for Nutritional Factors





© hari@sorghum.res.in

# Genetic Variability

| Fe (mg/kg) | Zn (mg/kg) | Corr. Fe-Zn | Sorghum material | Reported by |
|---|---|---|---|---|
| 20-37 | 13-31 | 0.55** | Hybrid parents, breeding lines (84) | Reddy et al. 2005 |
| 26-61 | 21-57 | 0.75** | Germplasm accn. (29) | Ashok Kumar et al. 2009 |
| 30-44 | 22-33 | 0.85** | Commercial cultivars (20) | Ashok Kumar et al. 2010 |
| 8-133 | 15-91 | 0.60** | Germplasm accn. (1394) | Reddy et al. 2010 |

**Grain Fe and Zn in selected white grain landraces (2262)**

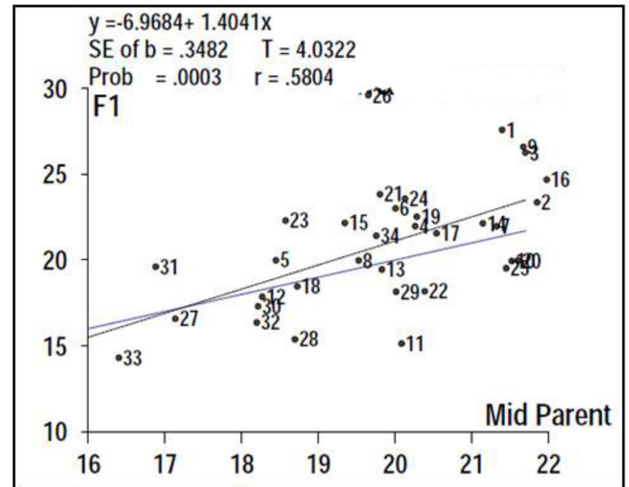
| IS No. | Fe (mg/kg) | Zn (mg/kg) | Origin | Race |
|---|---|---|---|---|
| 23680 | 71 | 44 | Mozambique | *Caudatum* |
| 5308 | 63 | 45 | India | *Guinea* |
| 5427 | 60 | 57 | India | *Durra* |
| 3790 | 58 | 54 | Taiwan | *Kafir-bicolor* |
| 3696 | 57 | 40 | Taiwan | *Guinea-bicolor* |
| 5514 | 56 | 45 | India | *Guinea-Bicolor* |

(Source: http://hdl.handle.net/11038/10081; Ashok Kumar, ICRISAT)

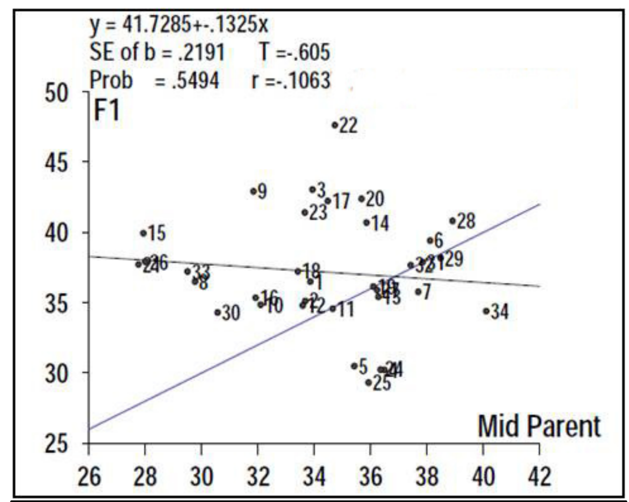
# Genetic Control of Grain Micronutrients

- Both additive and non-additive gene actions important
- Dominant gene action more predominant for grain Fe, while additive gene action predominant for grain Zn; Predictability ratio was 0.14 for grain Fe and 0.65 for Zn
- Scope for heterosis breeding for grain Fe, while for improving grain Zn progeny selection in pedigree breeding will be effective
- To develop hybrids with high grain Fe and Zn content both parents need to be improved for the micronutrients

| Source | Iron | Zinc |
|---|---|---|
| GCA | 22.0** | 34.2*** |
| SCA | 23.9*** | 6.6* |
| $\sigma^2$ A | 2.78 | 5.54 |
| $\sigma^2$ D | 17.20 | 2.95 |
| $\sigma^2$ gca / $\sigma^2$ sca | 0.08 | 0.94 |
| Av. Heterosis (%) | 6.78 | 4.55 |




Zn: mid-parent vs. $F_1$




Fe: mid-parent vs. $F_1$

# Agronomic-fortification

| | Fertilizer Treatment | Fe (ppm) | Zn (ppm) |
|---|---|---|---|
| 1 | RDF | 35.0 | 17.9 |
| 2 | RDF + 50 kg/ha $ZnSO_4$ as soil appl. | 34.7 | 16.9 |
| 3 | RDF + 50 kg/ha $FeSO_4$ as soil appl. | 38.7 | 18.1 |
| 4 | RDF + 50 kg/ha each $ZnSO_4$ + $FeSO_4$ as soil appl. *fb* foliar spray of $ZnSO_4$ at 0.5% and $FeSO_4$ at 0.1% at 40 DAS | 44.1 | 18.6 |
| | CD (0.05) | 11.8 | 14.6 |

Cultivars: M 35-1, Phule Maulee, Phule Yashoda, Phule Chitra, CSH 15R

- Soil type or micronutrient application have only limited influence on grain Fe and Zn when the soils are not deficient in these minerals
- Significant cultivar × year or genotype × environment (G × E) interactions have been reported for both grain Fe and Zn content
- Multi-location & multi-season evaluation is necessary for identifying stable donors for micronutrient enrichment breeding programmes
- As sorghum is grown in varied soil types with varying levels of fertility and nutrient management, it is necessary to assess the stability of grain micronutrients for biofortification

# Biofortification Target

Considering the level of sorghum consumption, nutrient retention in grain storage, milling and food preparation and nutrient bioavailability, HarvestPlus suggested a target of 70 mg/kg for Fe and 40 mg/kg for Zn

**Based on the extent of genetic variability observed among landraces**

| Cultivar/ germplasm | Grain Fe (mg/kg) |
|---|---|
| Target | 60 |
| Base level | 30 |
| GK 4035 & NSH 703 | 44 |
| ICSB 10 & ICSB 263 (Improved parents) | 48 |
| PVK 801 | 49 |
| IS 23680 | 71 |

| Cultivar/ germplasm | Grain Zn (mg/kg) |
|---|---|
| Target | 32 |
| Base | 20 |
| GK 4035 | 33 |
| ICSB 484 (Improved parents) | 32 |
| *Pacha Jonna* | 34 |
| IS 5427 | 56 |

(Source: Ashok Kumar, ICRISAT)

# Sorghum Biofortification Project

**Biofortifying Sorghum with high grain Iron and Zinc content for combating micronutrient malnutrition (funded by DBT; 2012-15)**

**Objectives**

- To develop new mapping populations for grain Fe and Zn contents using diverse parents
- To identify markers linked to high grain Fe and Zn content Quantitative Trait Loci (QTLs) for use in marker assisted selection (MAS)


**ICRISAT**
- –Ashok Kumar
- –Fred Rattunde
- –Santosh P Deshpande
- –B Ramaiah

**DSR , Hyderabad**
- -Hariprasanna K.
- -JV Patil

**VNMKV, Parbhani**
- –Shivaji P Mehtre


ज्वार अनुसंधान निदेशालय
Directorate of Sorghum Research
(An ISO 9001:2008 Certified Institute)

# Sorghum Biofortification - Progress

## HarvestPlus-ICRISAT (Feb. 2014)

- ICSH 14001 (Fe 49 ppm and Zn 38 ppm)
- ICSH 14002 (Fe 46 ppm and Zn 32 ppm)
- ICSA 661 × ICSR 196 (Fe 45 ppm and Zn 36 ppm)
- ICSA 318 × ICSR 94 (Fe 45 ppm and Zn 34 ppm)
- ICSA 336 × IS 3760 (Fe 45 ppm and Zn 40 ppm),
- R line/variety ICSR 14001 (Fe 42 ppm and Zn 35 ppm)

## Hytech Seed Company - Sorghum hybrid 3204 (July 2014)

- Dual season (*kharif* and late *rabi*)
- Dual purpose (grain and dry fodder)
- Tall with bold shiny white grains
- Fe - 46 mg/kg
- Zn - 29 mg/kg

# The African Biofortified Sorghum (ABS) Project

[http://biosorghum.org/]

Dr. Florence Wambugu; CEO, Africa Harvest and Coordinator, ABS Project
Dr. Paul Anderson: PI, Exec. Dir. of International Programs, Donald Danforth Plant Science Center

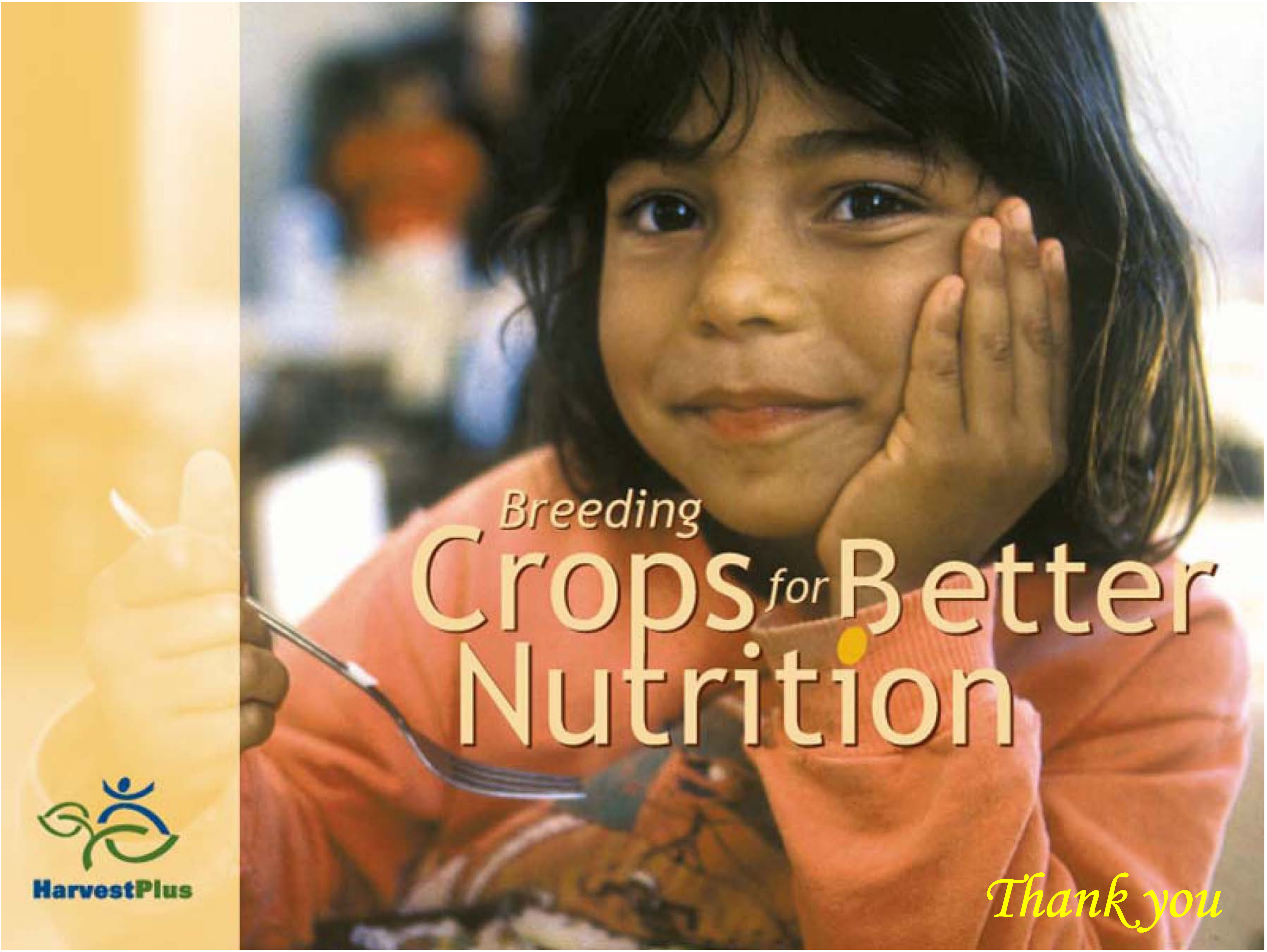
- **A joint project of a consortium consisting of Africa Harvest and 7 other agencies and Pioneer-Dupont (2005)**
- **Funding from the Bill & Melinda Gates foundation with a budget of $18.6 million over five years**

Project Funded By BILL & MELINDA GATES foundation

- **Mission - to develop a more nutritious and easily digestible sorghum, that contains increased levels of essential amino acids, especially lysine, increased levels of Vitamins A and E, and more available Fe and Zn, for the arid and semi-arid tropical areas of Africa**

# Conclusion

- Sorghum biofortification can be a feasible strategy
- Offers a long-term, sustainable, food-based solution
- Targets resource-poor, micronutrient-deficient people in remote rural areas
- One-time investment to develop seeds that fortify themselves
- Requires a multidisciplinary research approach
- Adequate genetic variation in Fe & Zn contents has been detected; genetic control has been established


Breeding
Crops for Better
Nutrition
HarvestPlus
Thank you