

"Plant Biotechnology for Sustainability and Global Economy"

Oeiras, April 13th 2016

Plants for food and health: biofortification

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Escola Superior de Biotecnologia

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Planlech



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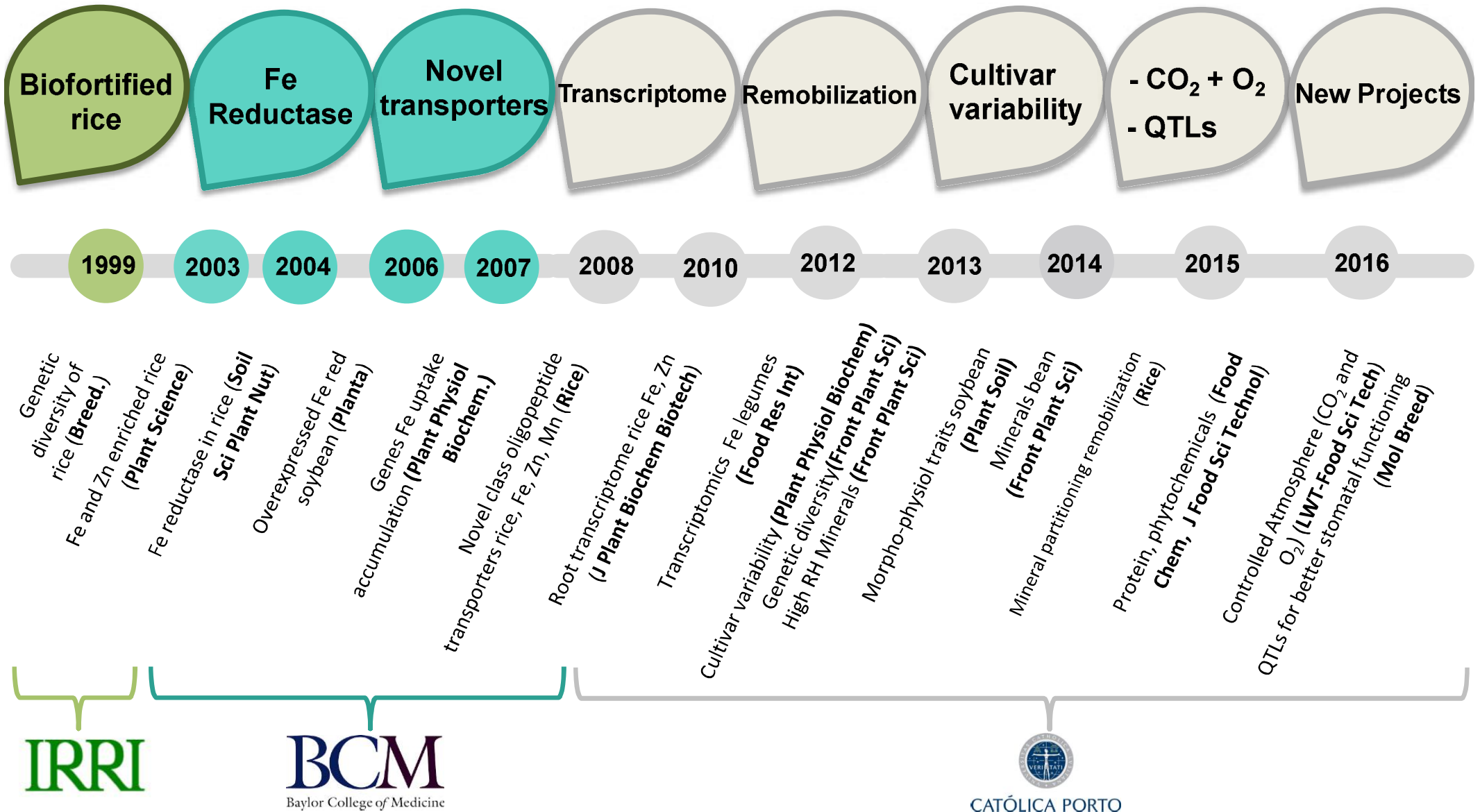
Who am I



- Plant Biologist and Plant Geneticist
- Assistant Professor at Faculty of Biotechnology, Catholic University of Portugal
- Chief Editor of Frontiers in Plant Nutrition
- Head of the PlanTech lab

My passion: understand how nutrients are taken up or synthesized in plant foods and to understand **how we can “trick” plants into storing more of these nutrients in their edible organs.**

My "expertise"



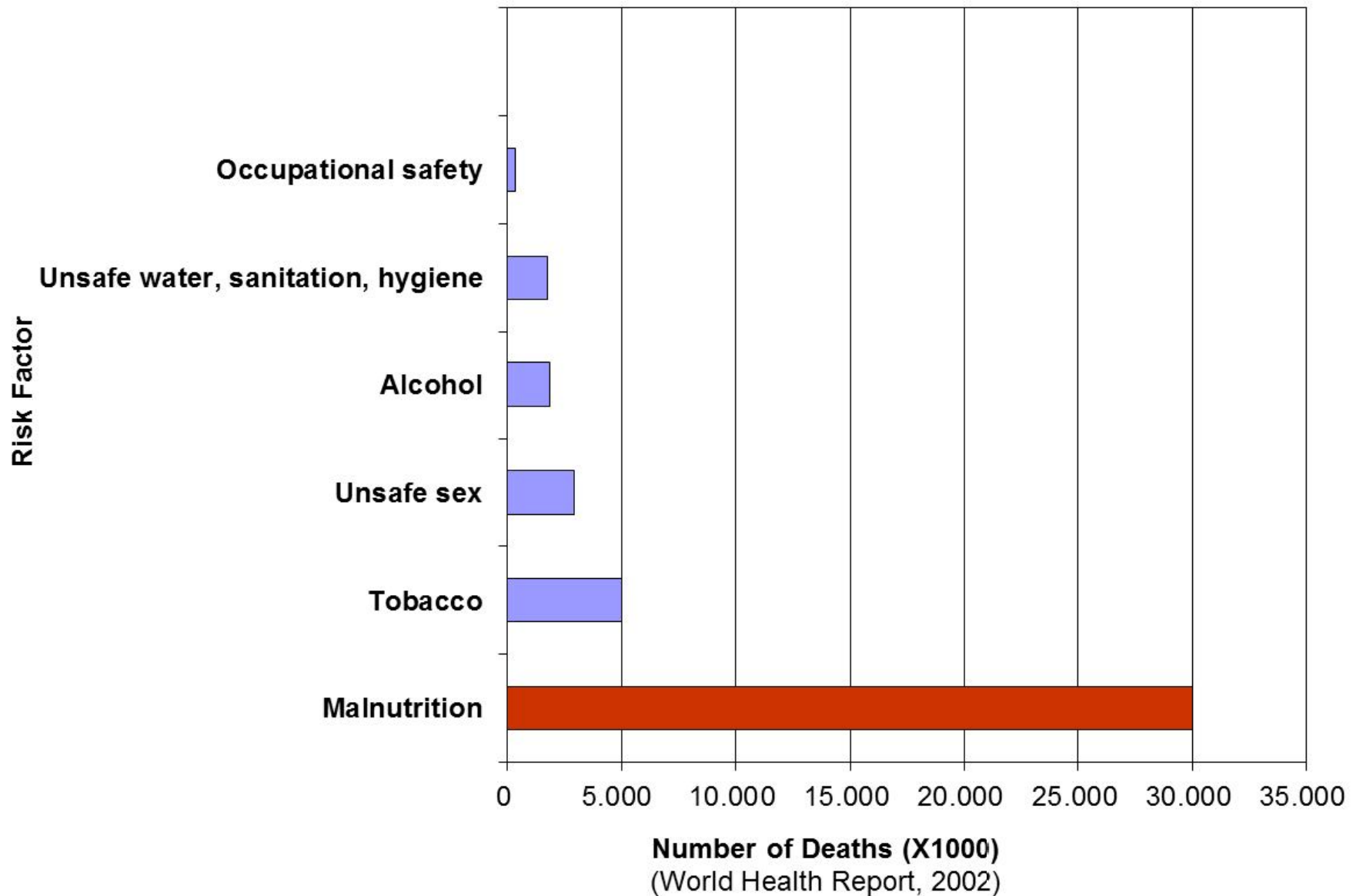


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Essential nutrients for proper health

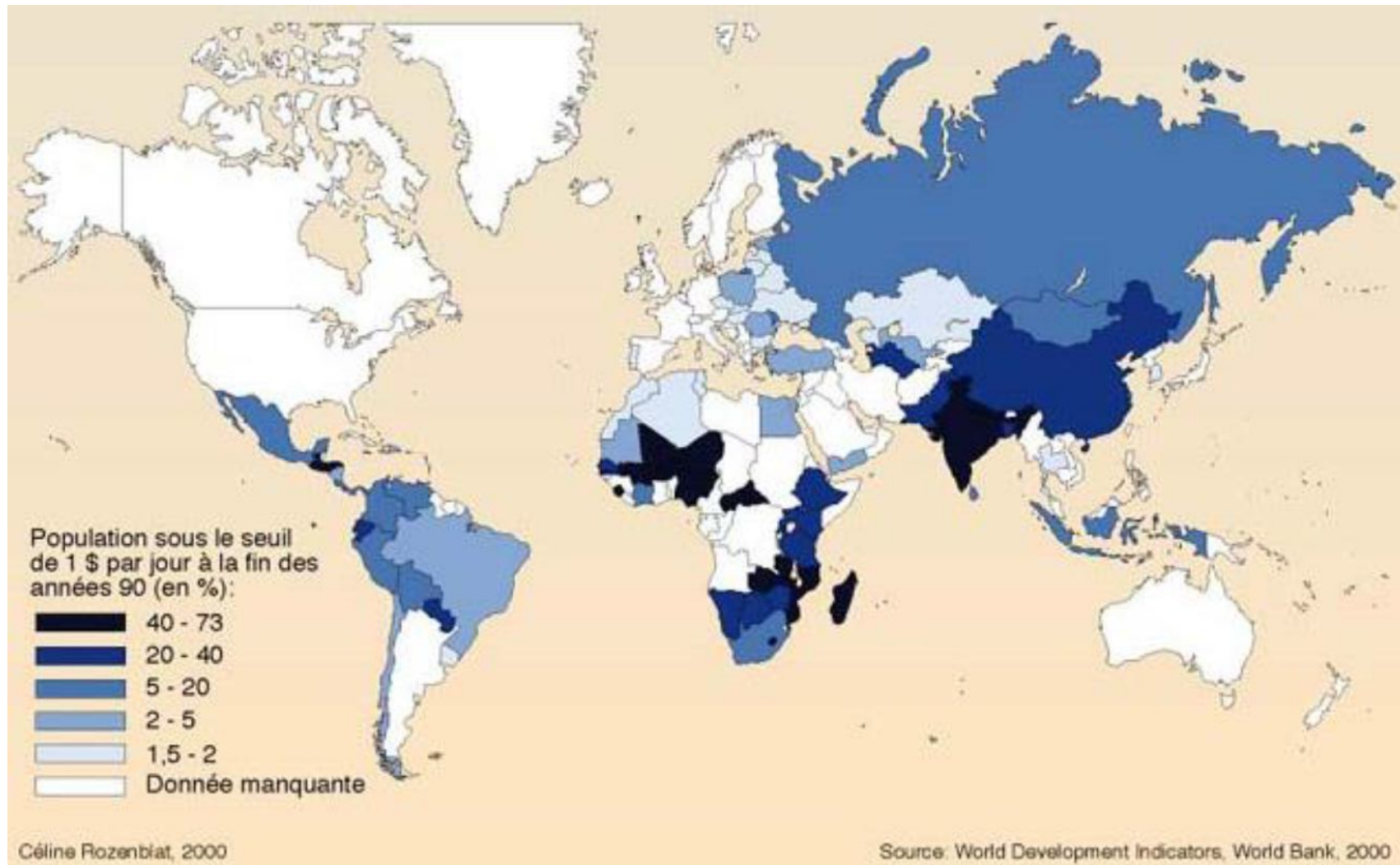
Vitamins		Minerals		
<i>Water soluble</i>	<i>Fat soluble</i>	<i>Macrominerals (100 mg or more)</i>	<i>Microminerals (<15 mg)</i>	<i>Ultra-trace minerals(µg)</i>
Vitamin B6	Vitamin A	P	Zn	Cu
Vitamin B ₂	Vitamin D	Ca	Fe	I
Vitamin B ₁	Vitamin K	Mg	S	Mo
Vitamin B ₁₂ -Niacin	Vitamin E		F	Se
Vitamin C			Mn	Cr
Biotin			B	B
Vitamin B ₃			K	Co
Folic acid				

WHO Major Risk Factors

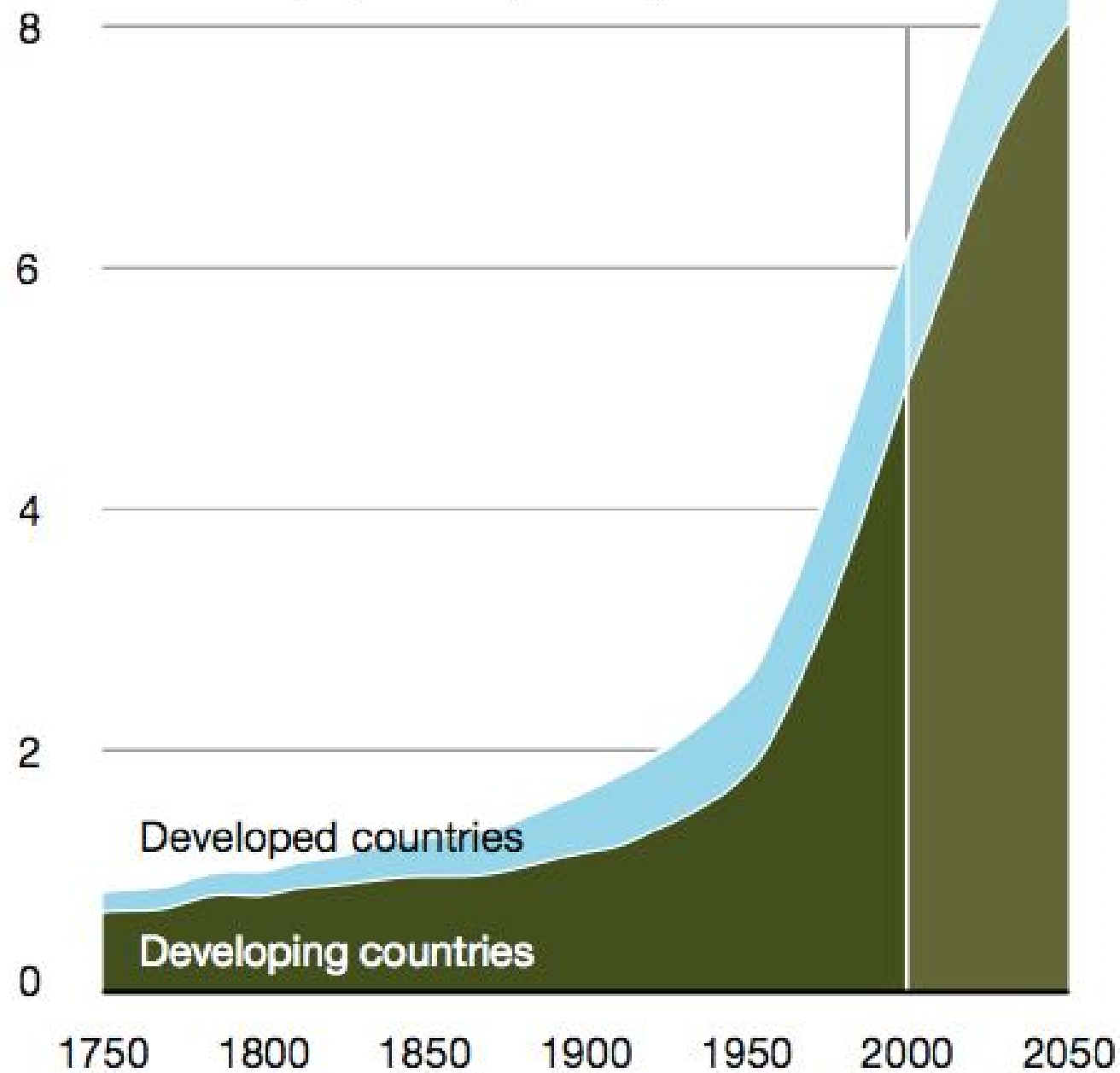


Malnourishment leads to \approx 30 million deaths worldwide(1/s)

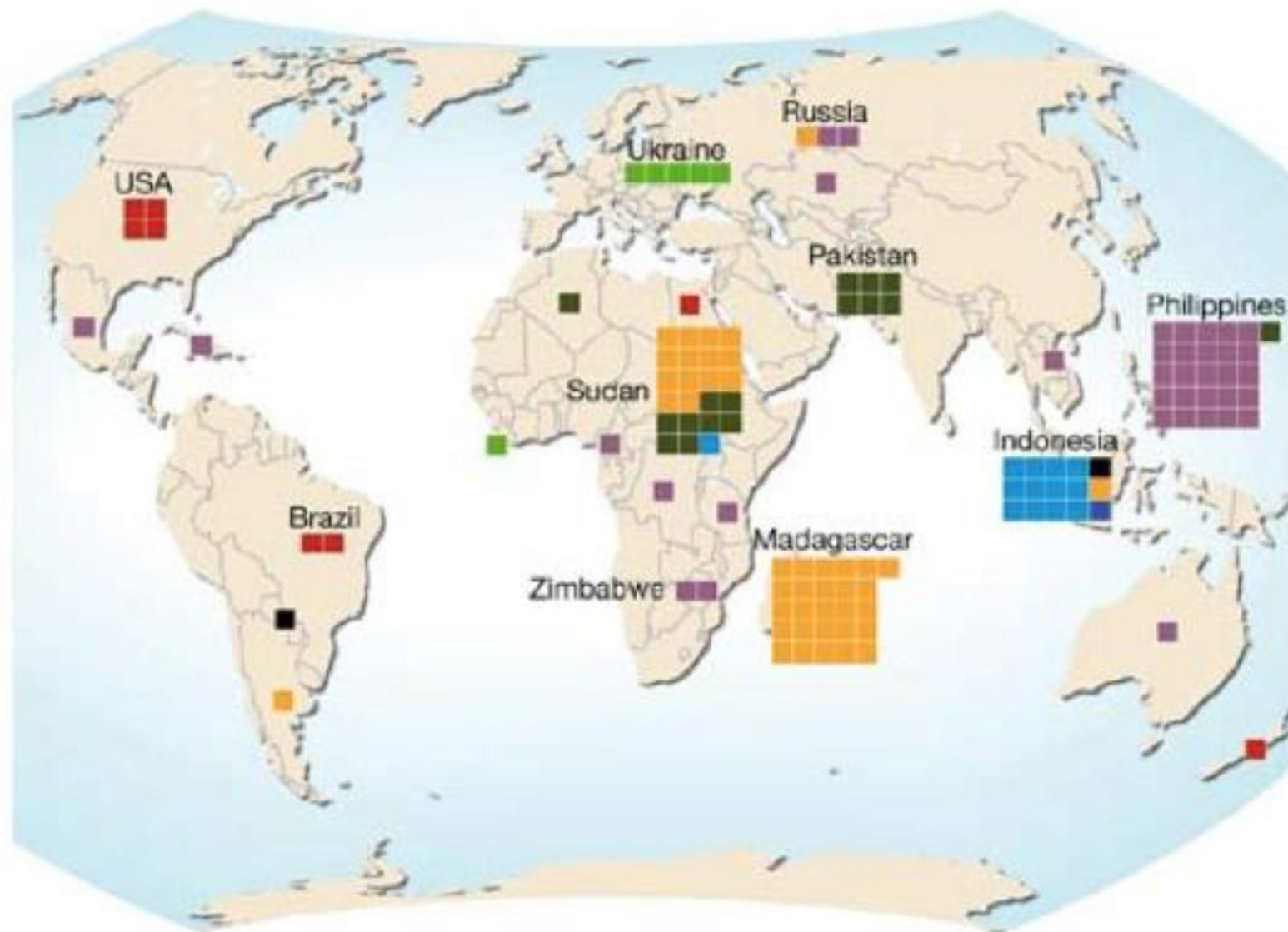
Poverty line: % population living with less than 1 USD per day



Global population, estimates and projections (billions)



Fonte: FAO data and projections



Agricultural international land leases

South Korea	2 000
China	1 500
UAE	710
Saudia Arabia	620
Japan	320
Libya	250
Malaysia	40
India	10

thousand hectares

Each square represents 50 000 hectares. Values under this value are represented with one square.

Strategies to improve nutrition (The World Bank)

Short routes

- | | | |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none">• Community-based nutrition and health services (community growth promotion programs, community Integrated Management of Childhood Illnesses [C-IMCI])• Facility-based nutrition and health services (health and nutrition services, and antenatal care)• Micronutrient supplements• Micronutrient fortification• Targeted food aid• Biofortification | <ul style="list-style-type: none">• Conditional cash transfers• Microcredit cum nutrition education• Food supplementation• Micronutrient supplements• Food stamps• Targeted food aid | <ul style="list-style-type: none">• Maternal nutrition, knowledge, and care-seeking during pregnancy and lactation• Infant and young child feeding• Weight control education• Hygiene education• Promoting healthy life styles (increase physical activity; consume more fruits and vegetables and less salt, sugar, and fat, and so on) |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|



Biofortification

Fortification:

- Nutritional enrichment of foods during harvesting or processing

Biofortification:

- Utilization of improved varieties or improved practices that led to better uptake, partitioning or synthesis of essential nutrients during plant growth, so that the harvested products are themselves nutritionally richer

Biofortification

A sustainable strategy for nutritional enrichment!



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Micronutrient deficiency

Fe: 33 to 50% of population is at risk.

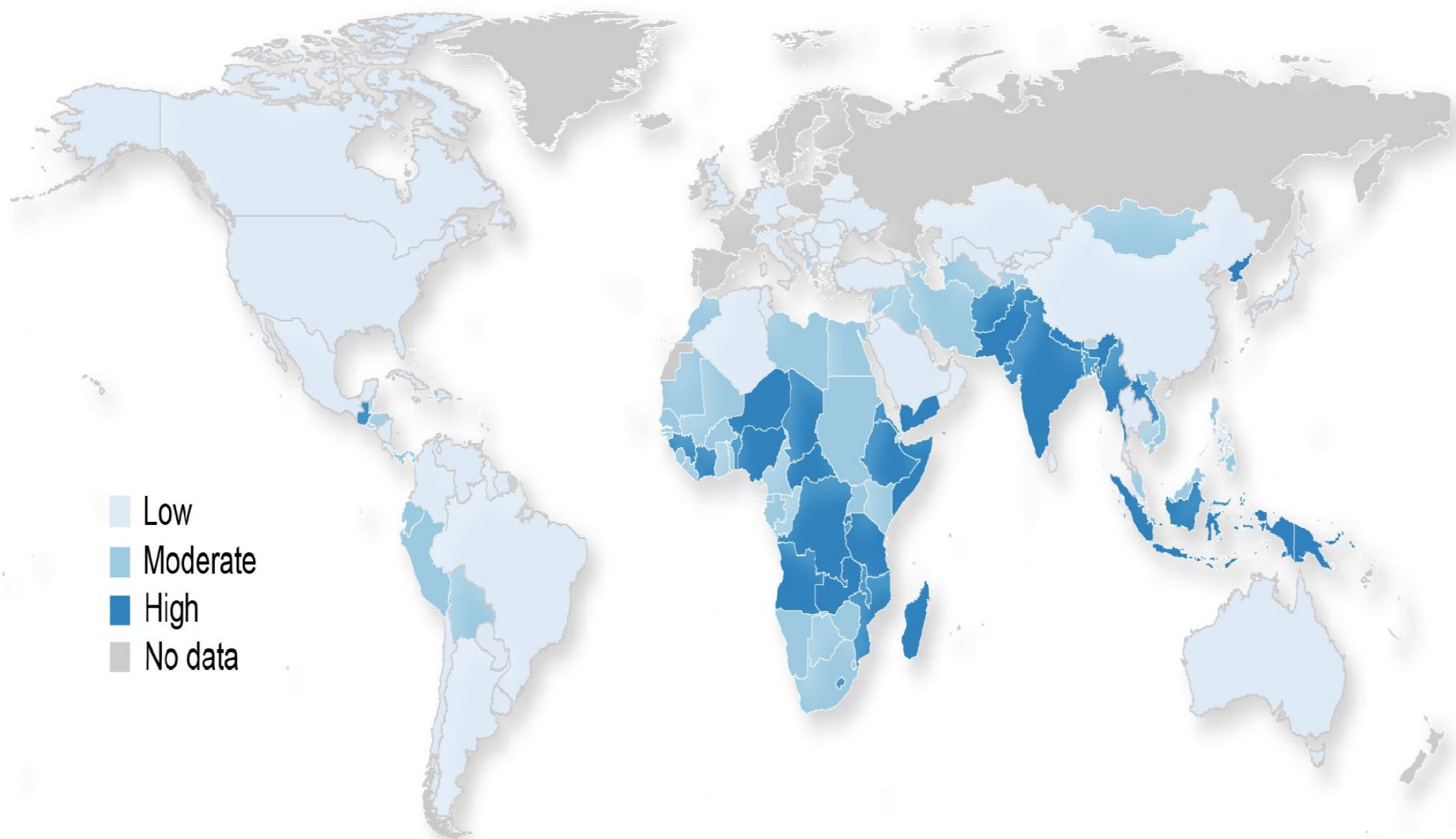
Zinc: ?????????? (same as Fe)

Vitamin A: 250 million

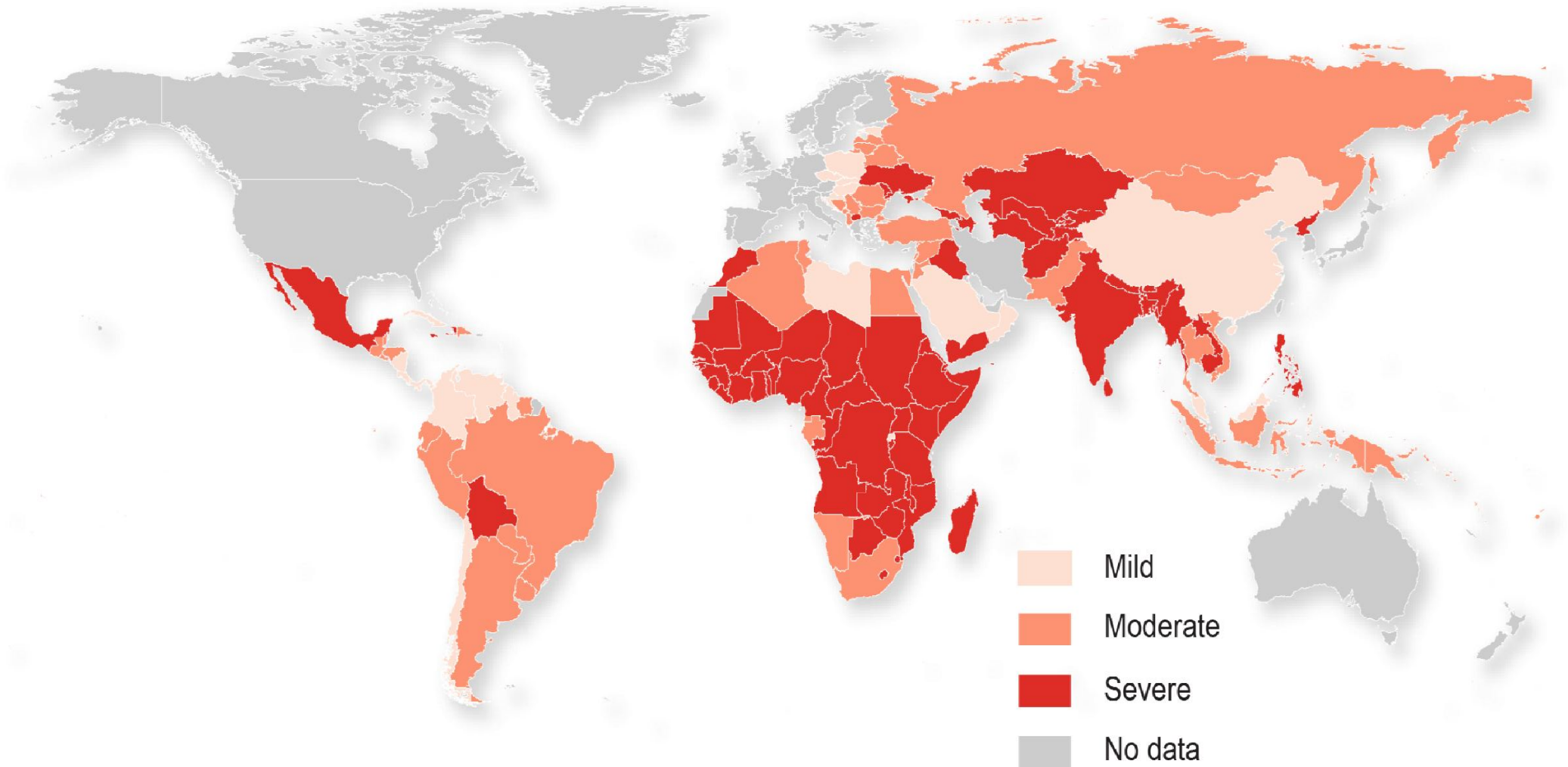
Iodine: 2 billion (1/3 school age children)



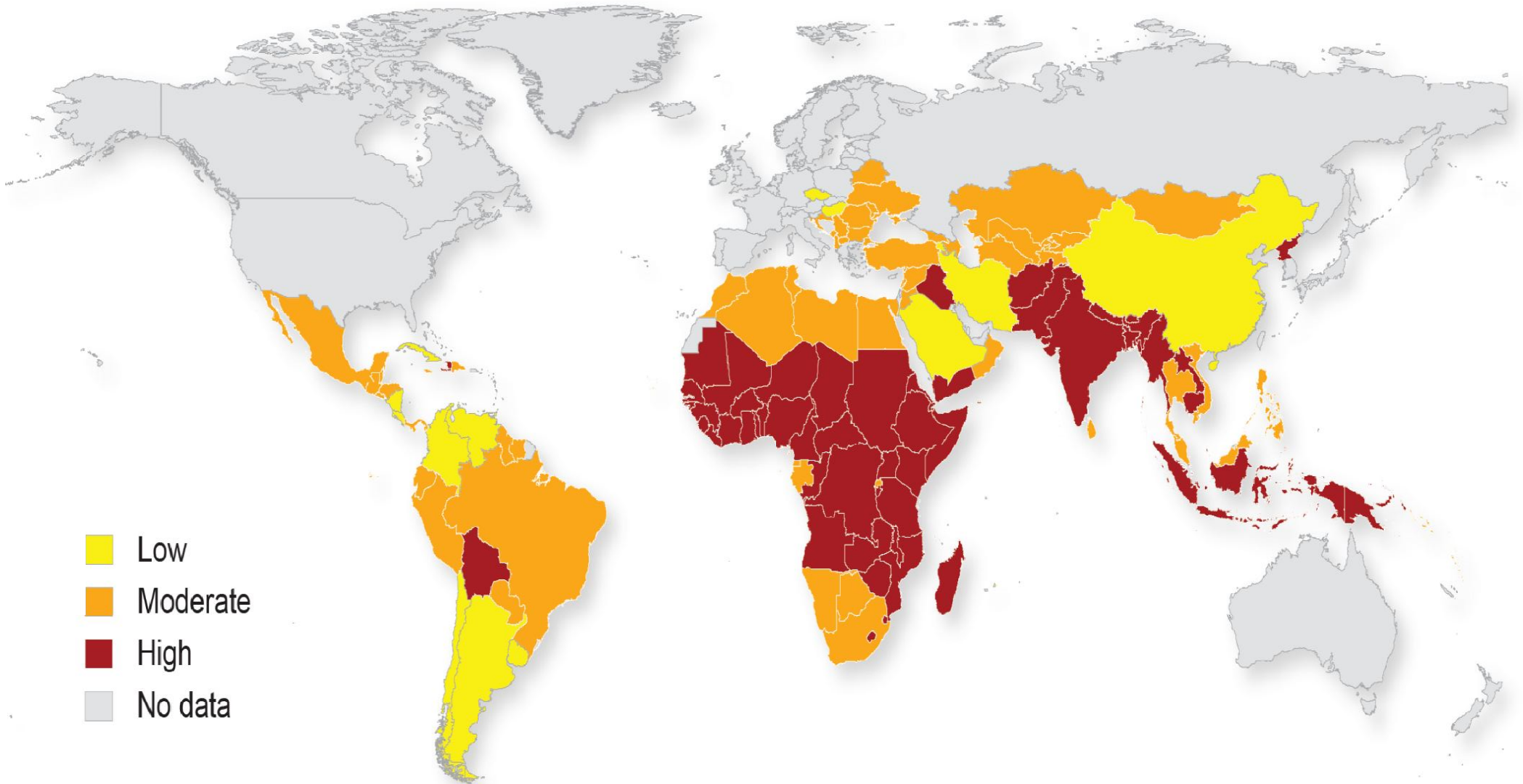
Prevalence of malnutrition: Zn



Prevalence of malnutrition: Vit A



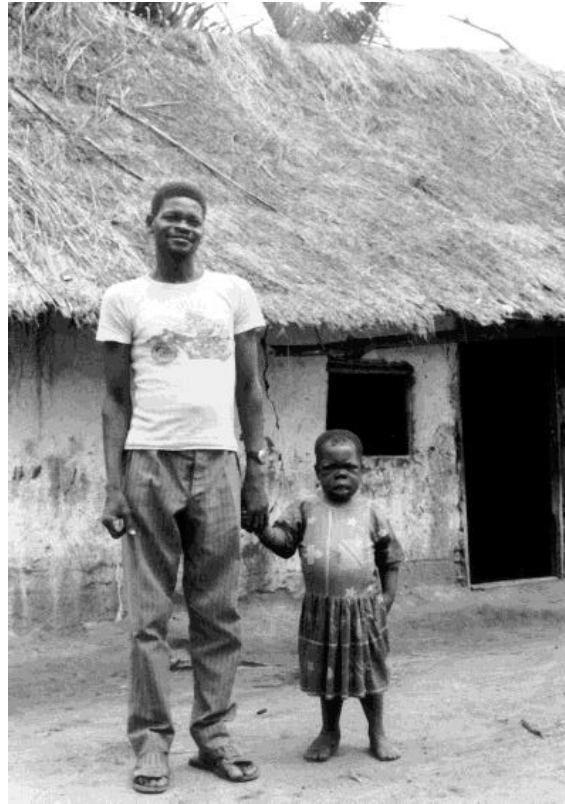
Prevalence of malnutrition: global



“Hidden hunger”



Zn



Iodine



Fe



Vitamin A



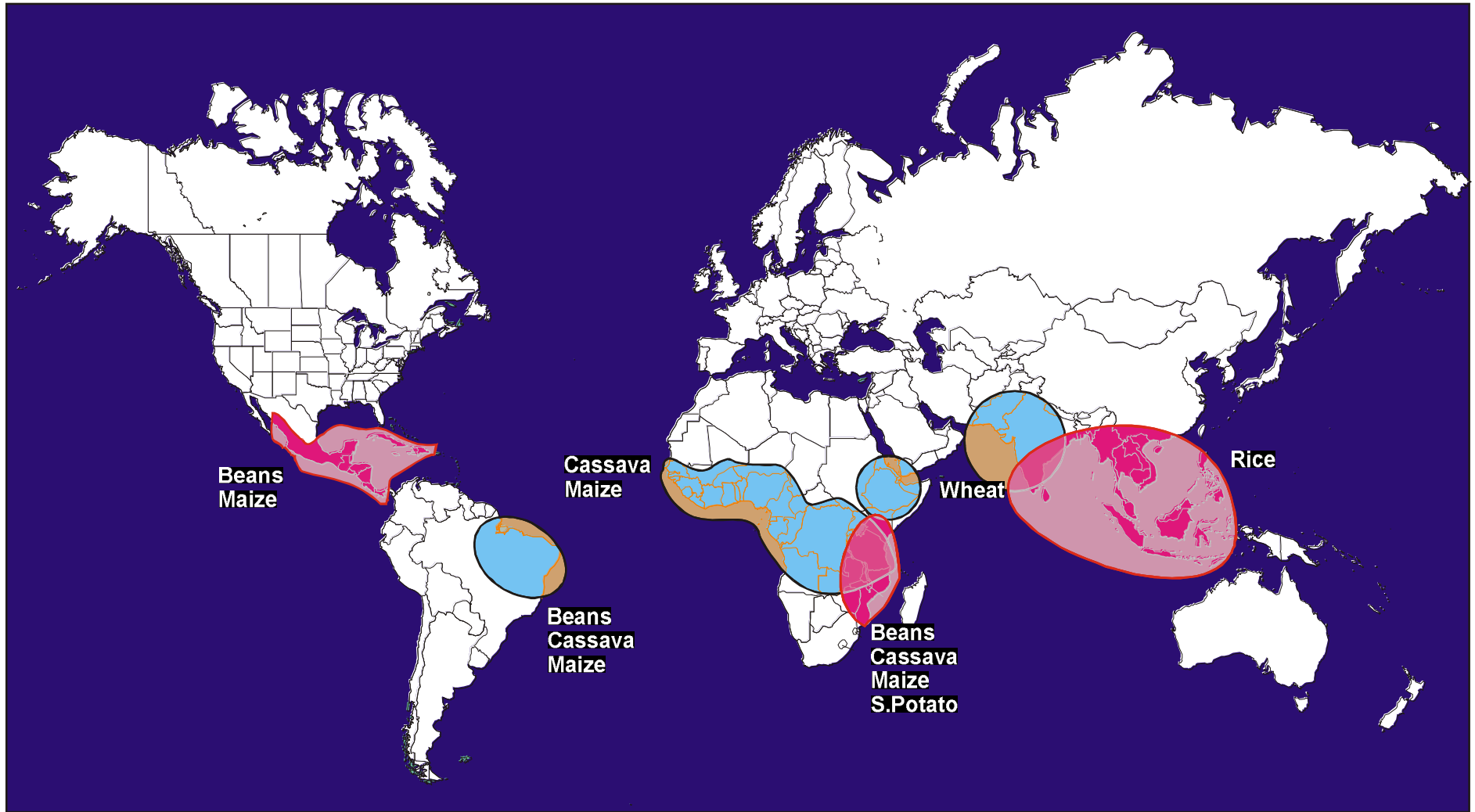
Ca

Biofortification

- 1. Conventional breeding**
- 2. Genetic screening (biodiversity)**
- 3. Bioavailability**
- 4. Genetic engineering**
- 5. Improved agricultural practices**



Biofortification: Impact



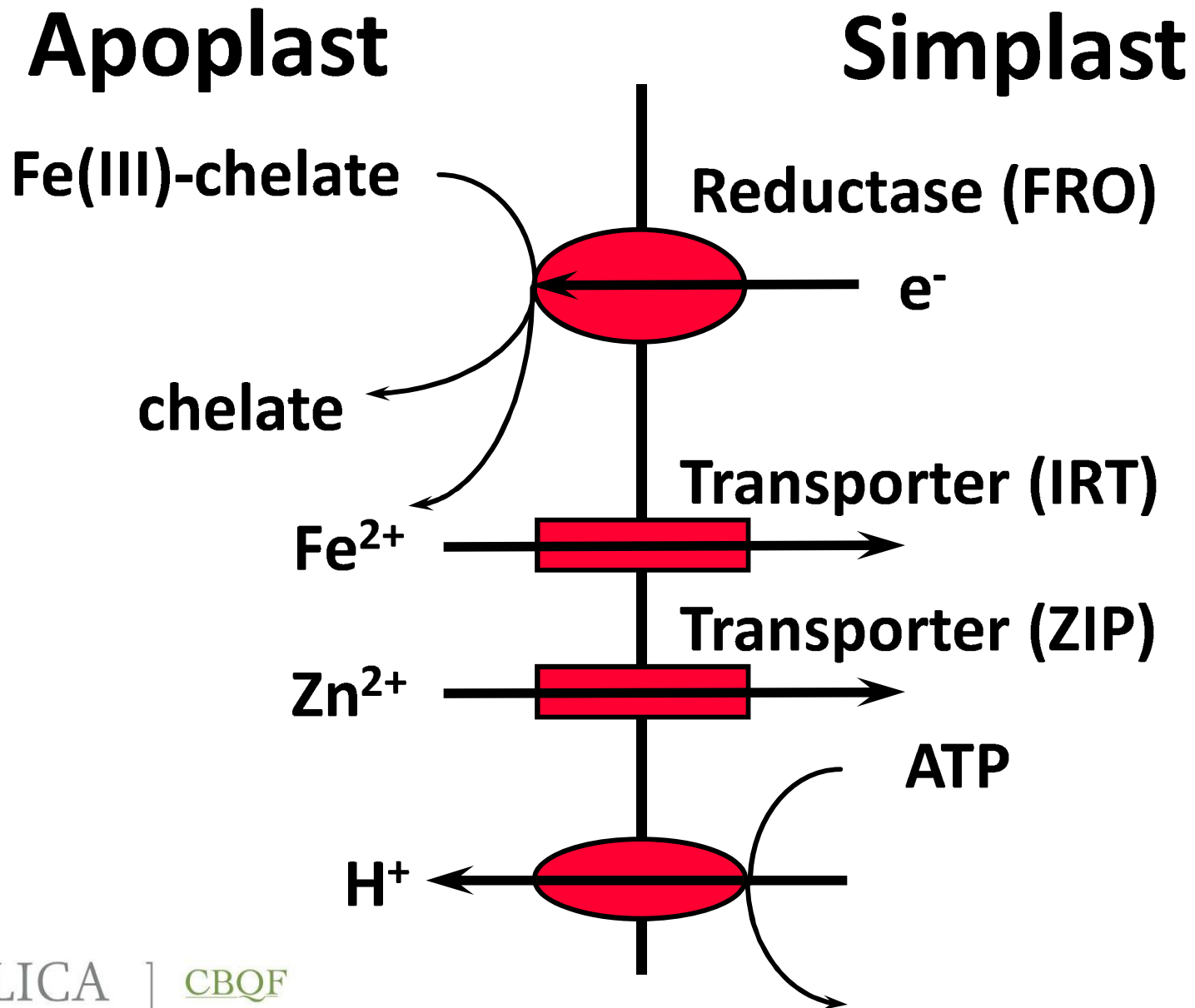
Staple foods

	Fe Conc. (mg/kg)	Zn Conc. (mg/kg)	Beta-carotene (mg/kg)
Brown rice	6 - 25	14 - 59	0 - 1
White rice	1 - 14	14 - 38	0
Wheat	10 - 99	8 - 177	0 - 20
Maize	10 - 63	12 - 58	0 - 10
Bean	34 - 111	21 - 54	0
Cassava	4 - 76	3 - 38	1 - 24

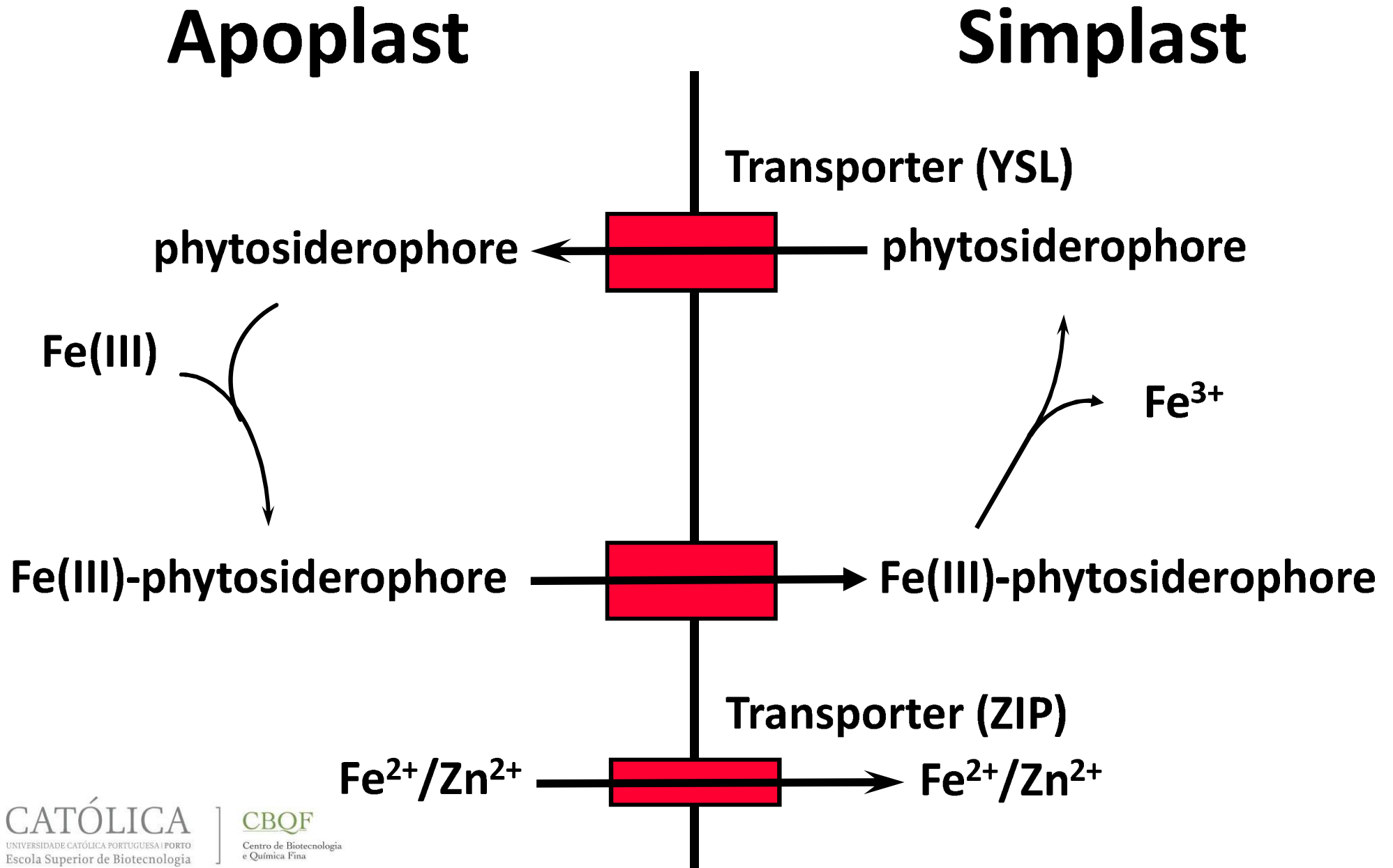
**What are some of the
metabolic processes and
how can we manipulate
them?**



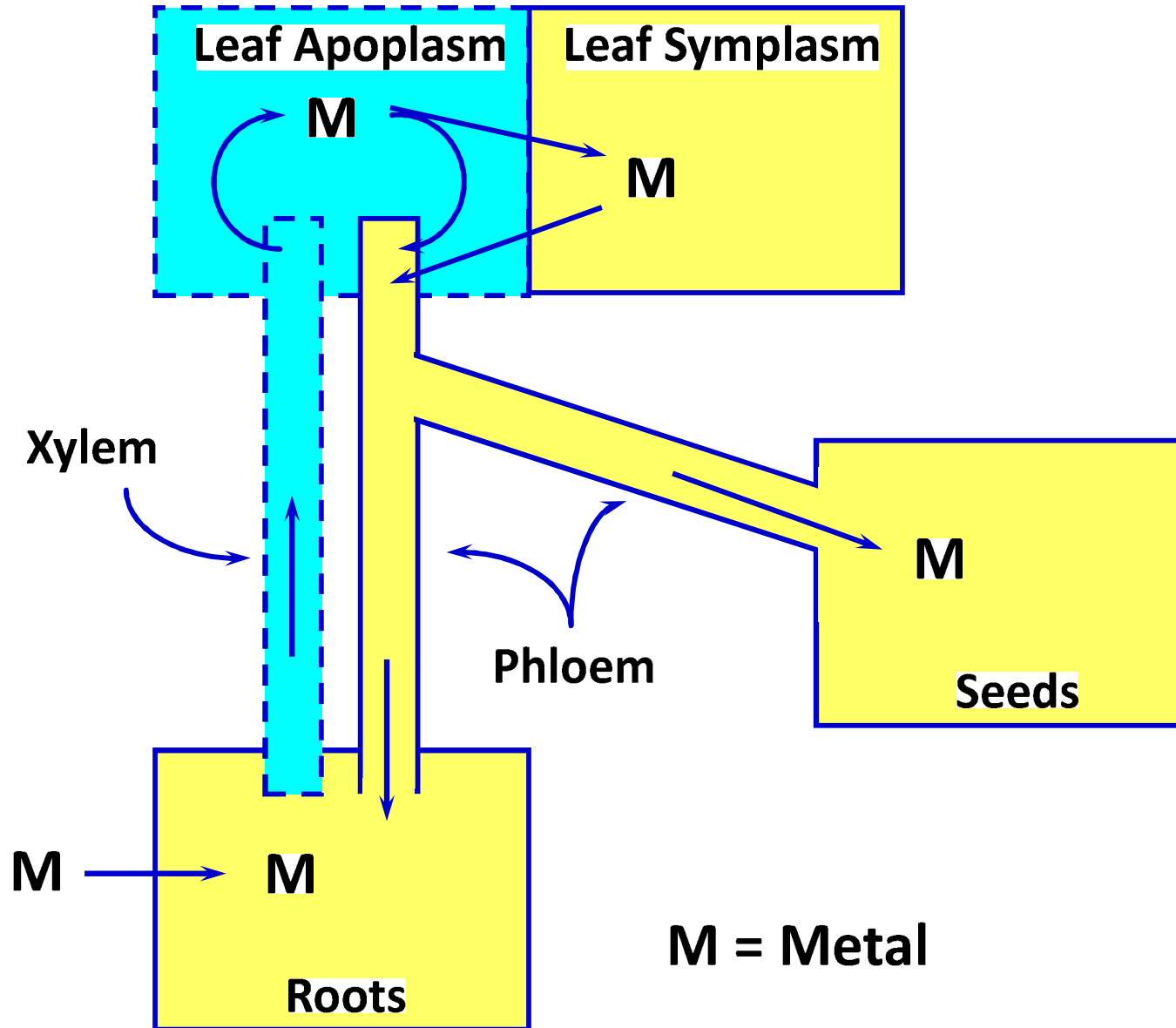
Fe acquisition (Dicot)

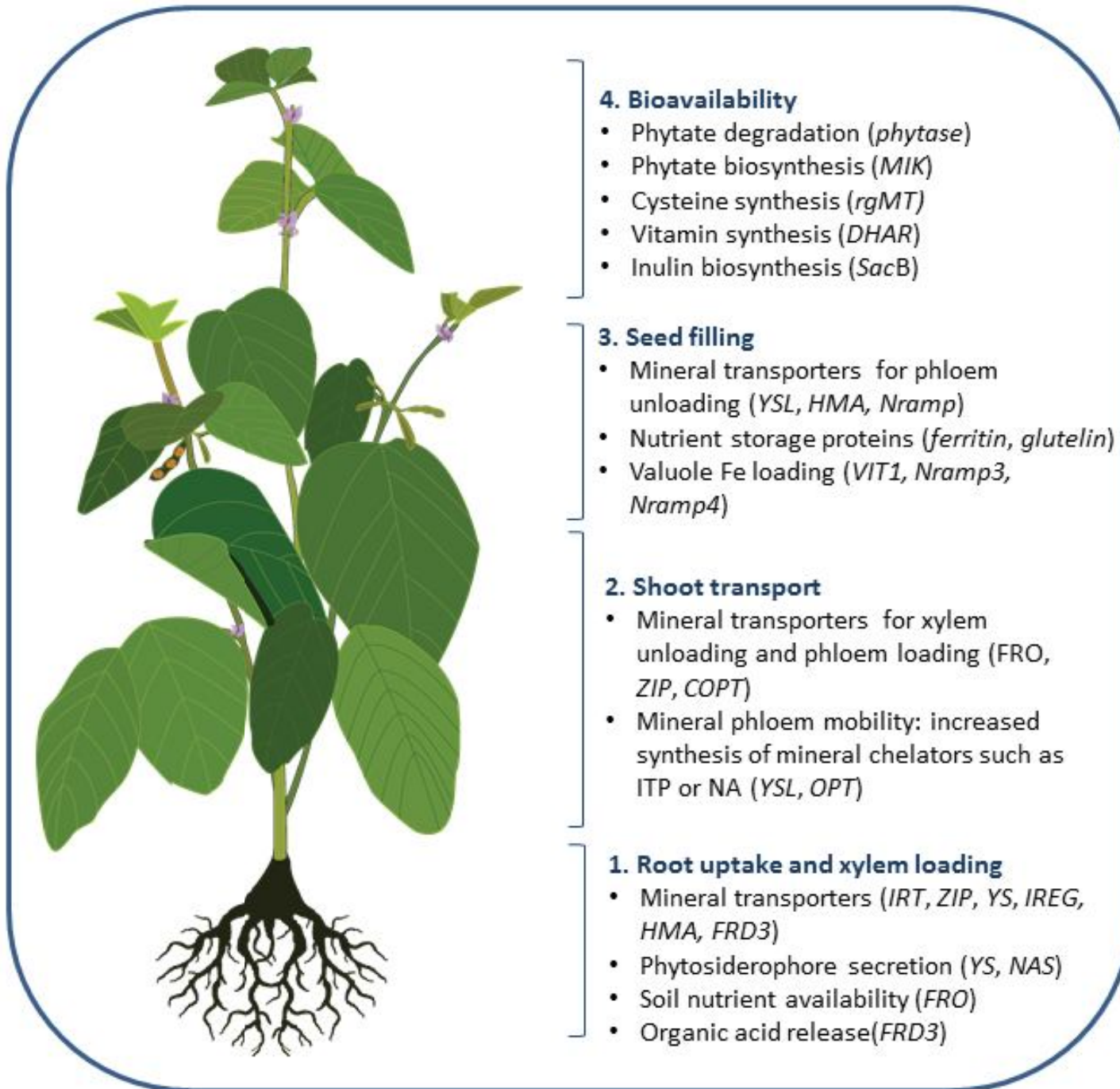


Fe acquisition (monocot)



Mineral transport in the plant



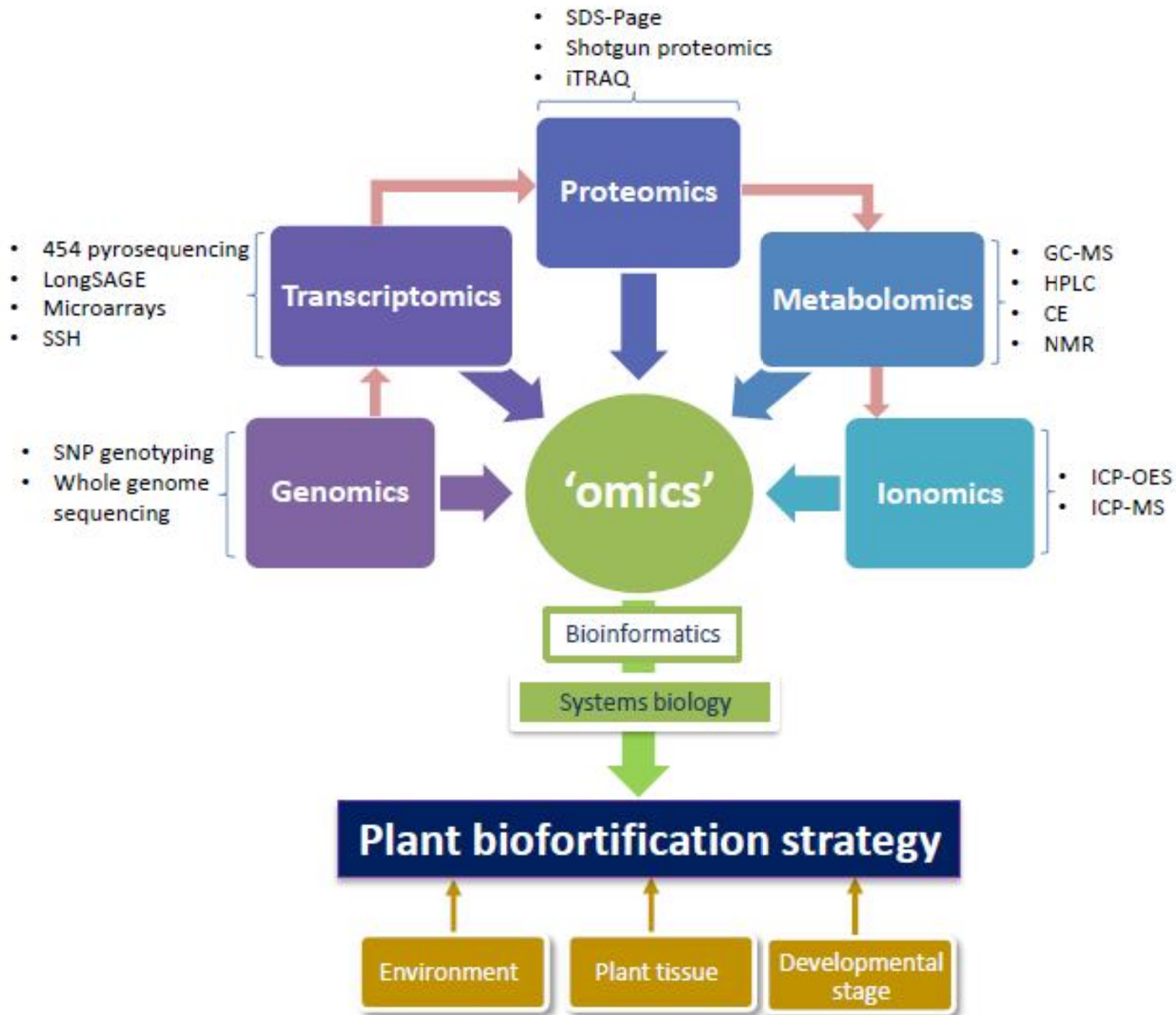


Importance of bioavailability

- **Bioavailable portion is as important as total amount**
- **Most staple foods (cereals, legumes) have low bioavailability (5% for Fe) due to antinutritional factors (phytates, poliphenols, etc)**
- **Example: increasing bioavailability from 5% to 30% is the same as augmenting 6x the total Fe amount**

Efficient biofortification strategy:

1. Accumulation in the edible plant part
2. Maintenance of values after harvesting and storage
3. Maintenance of plant productivity
4. Improved levels of nutrient in the organism (bioavailability)
5. No adverse side effects (e.g. allergens)



Biofortification examples

Crop	Improved nutritional trait	References
Rice	Beta-carotene	(Ye et al., 2000; Paine et al., 2005)
	Folate	(Storozhenko et al., 2007)
	Alpha-tocotrienol	(Zhang et al. 2012)
	Protein/amino acid	(Katsube et al., 1999; Lee et al., 2001; Tozawa et al., 2001; Lee et al., 2003; Wu et al., 2003;)
	Fatty acids	(Anai et al., 2003)
	Starch	(Terada et al., 2000, 2002; Itoh et al., 2003; Liu et al., 2003; Zhu et al., 2003; Tanaka et al., 2004; Chiang et al., 2005)
	Flavonoids	(Shin et al., 2006)
	Fe	(Goto et al., 1999; Lucca et al., 2001; Vasconcelos et al., 2003; Qu et al., 2005; Masuda et al., 2008, 2009, 2012; Ye et al., 2008; Lee and An, 2009; Lee et al., 2009, 2012; Wirth et al., 2009; Johnson et al., 2011)
Wheat	Zn	(Vasconcelos et al., 2003; Masuda et al., 2008, 2009; Lee et al., 2009; Johnson et al, 2011)
	Zn	(Cakmak et al., 2010)
	Low phytate	(Brinch-Pederson et al., 2000)
Soybean	Fe	(Borg et al., 2012)
	Fatty acids	(Chen et al., 2006; Eckert et al., 2006)
	Protein/amino acids	(Falco et al., 1995; Maughan et al., 1999; Dinkins et al., 2001; Li et al., 2005)
Maize	Tocopherol	(Eenennaam et al., 2003; Tavva et al., 2007)
	Isoflavone	(Yu et al., 2003)
	Beta-carotene	(Naqvi et al., 2009)
	Ascorbic acid	(Chen et al., 2003; Naqvi et al., 2009)
	Folate	(Naqvi et al., 2009)
Potato	Vitamin E	(Cahoon et al., 2003)
	Protein/amino acid	(Lai and Messing, 2002; Yang et al., 2002)
	Iron	(Drakakaki et al., 2005; Aluru et al., 2011)
	Anthocyanin	(Lukaszewicz et al., 2004)
	Aminoacids	(Zeh et al., 2001; Li et al., 2001)
Cassava	Carotenoids	(Ducreux et al., 2005; Diretto et al, 2006, 2007; 2006; Zain, 2010)
	Zeaxanthin	(Römer et al., 2002)
	Carbohydrate	(Schwall et al., 2000; Hellwege et al, 1997, 2000)
Canola	Protein	(Narayanan et al., 2011; Leyva-Guerrero et al., 2012)
	Iron	(Leyva-Guerrero et al., 2012)
Lettuce	Fatty acids	(Dehesh et al., 1996)
	Carotenoids	(Shewmaker et al., 1999)
Carrot	Folate	(Nunes et al., 2009)
	Resveratrol	(Liu et al., 2006)
	Iron	(Gotto et al., 2000)
Tomato	Calcium	(Park et al, 2004; Morris et al., 2008)
	Antioxidants	(Muir et al., 2001; Nigwegg et al., 2004; Giovinazzo et al. 2005; Butelli et al., 2008)
	Carotenoids	(Fraser et al., 2001, 2002; Mehta et al., 2002; Apel and Bock, 2009)
	Poliamines	(Mattoo et al., 2007)
	Folate	(Díaz de la Garza et al., 2004, 2007)
	Isoprenoids	(Enfissi et al., 2005)

Examples

- Rice (Fe e Zn)
- Rice(Vit A)
- Soybean (Fe)
- Others





Increasing Fe and Zn in transgenic rice



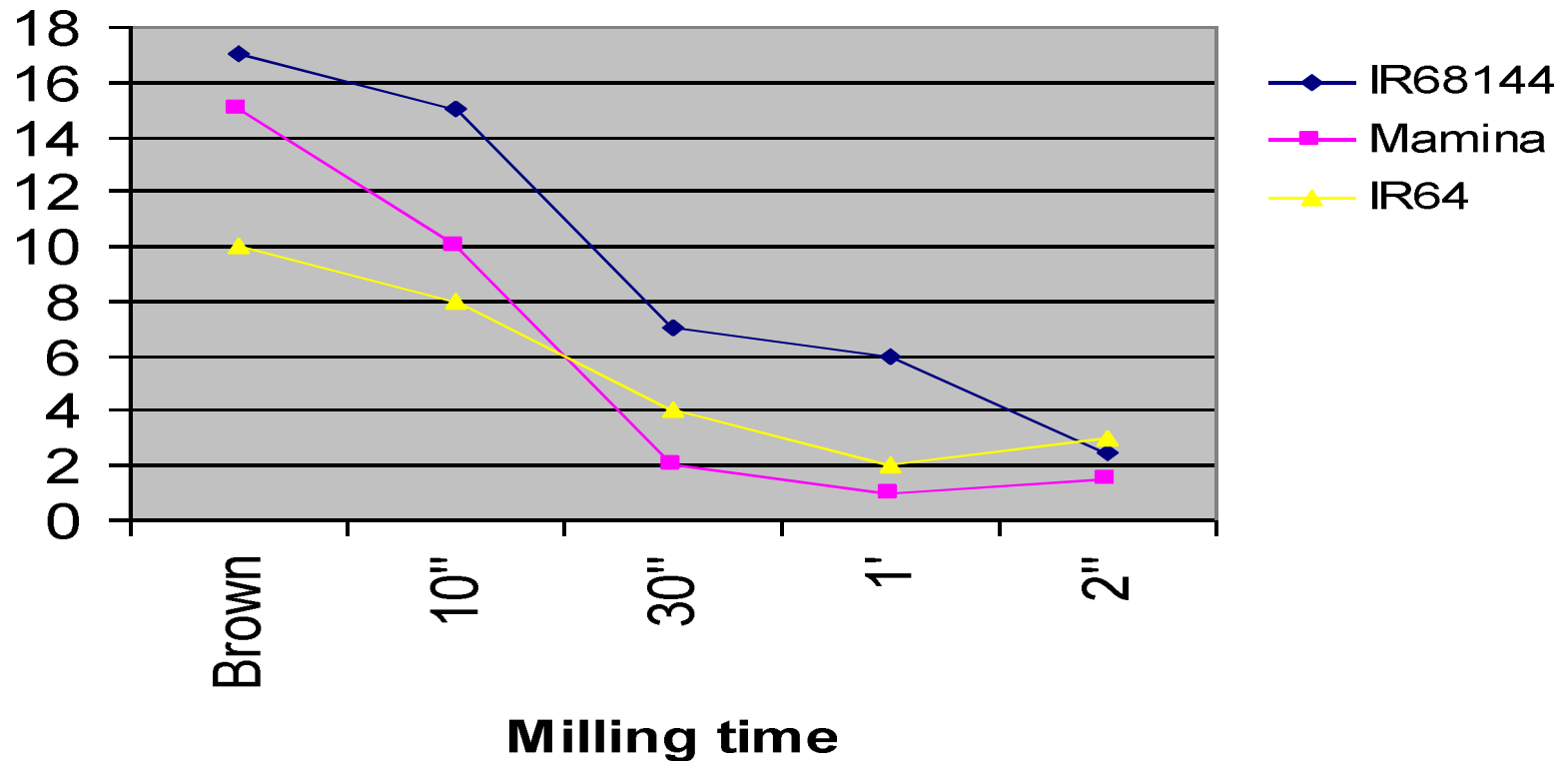
Rice (*Oryza sativa* L.)

- World's most consumed food (2/3 of the population)
- Grows in many types of habitat

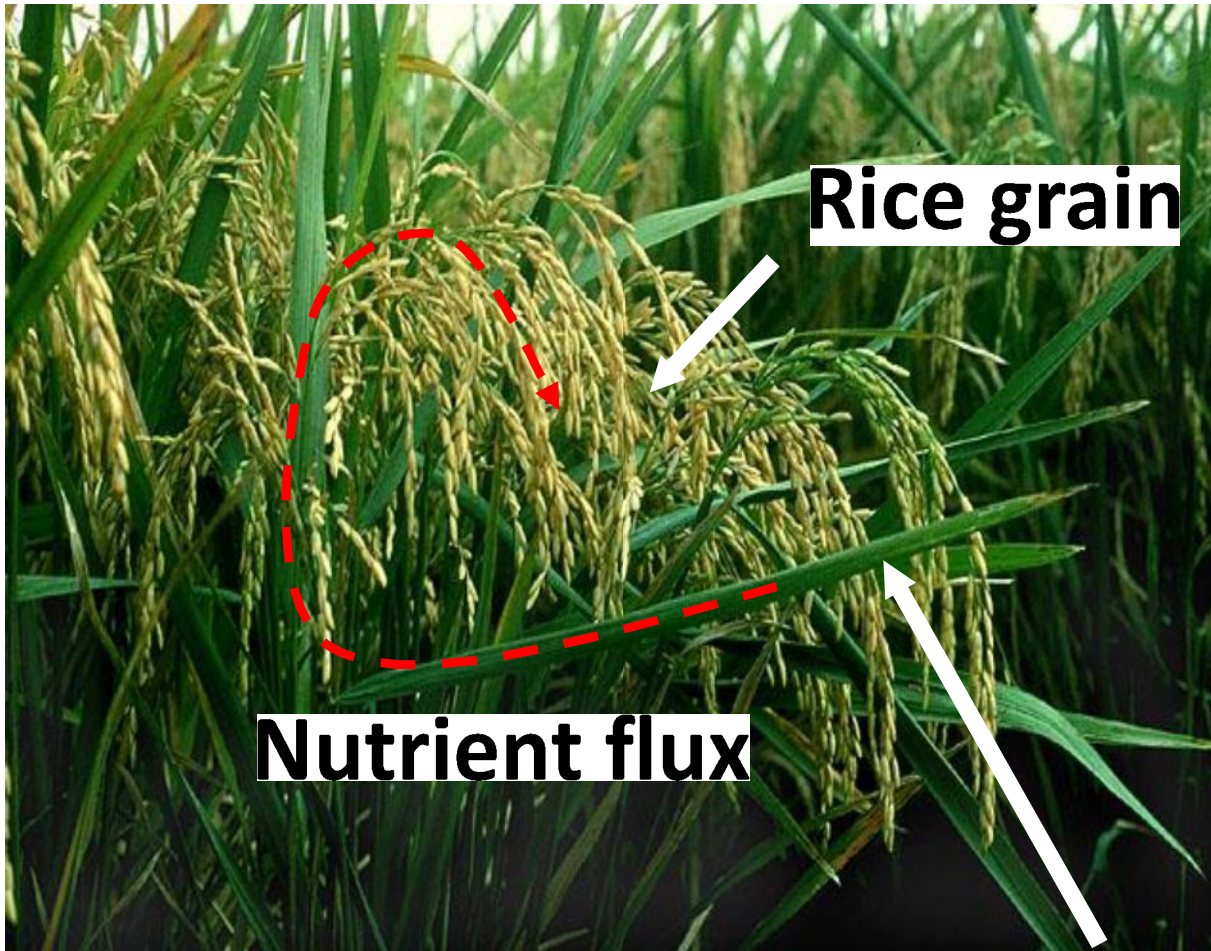


The problem

■ Rice is poor in Fe



Nutrient transport to the grain



Fe is mobilized to the seeds along with sugars, amino acids and others, via phloem

Flag leaf

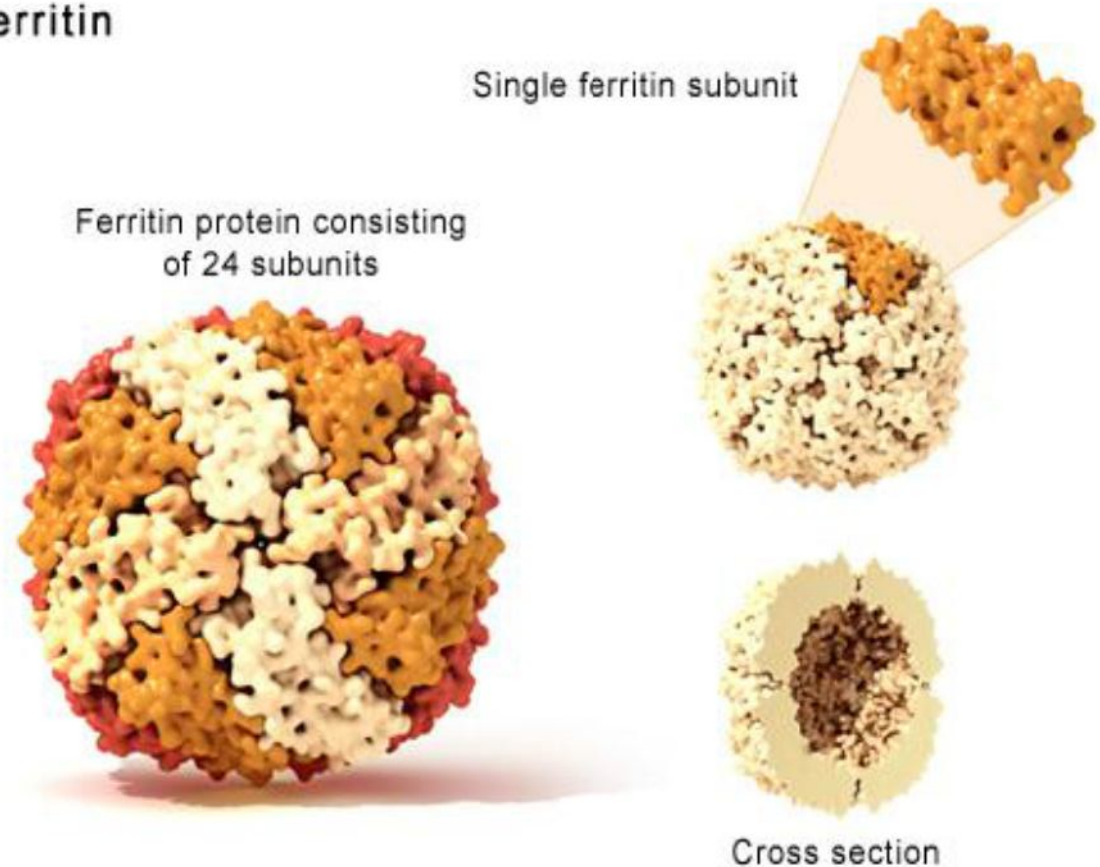


Ferritin

Strategy

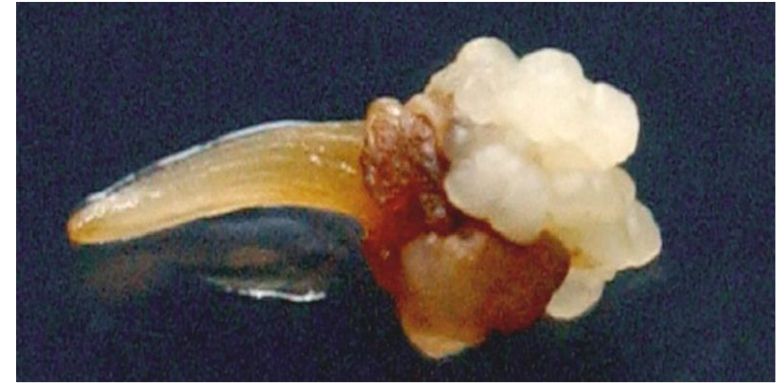
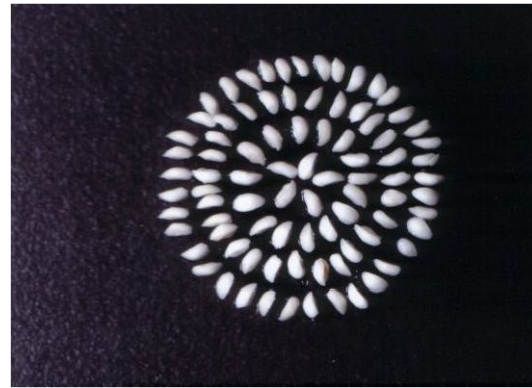
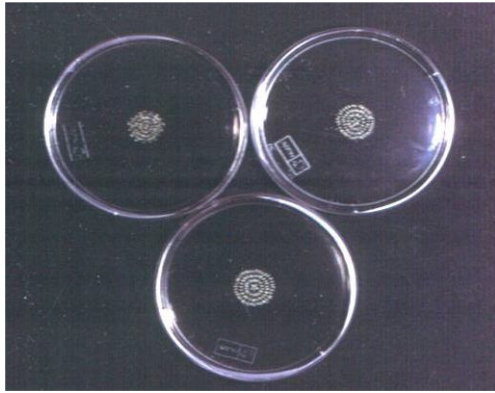
1. Variety
2. Transformation
3. Homozigots
4. ICP

Ferritin

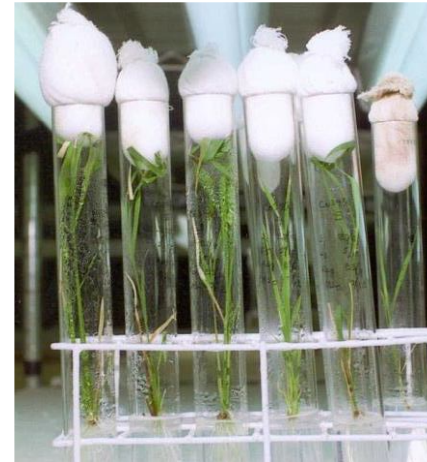


U.S. National Library of Medicine





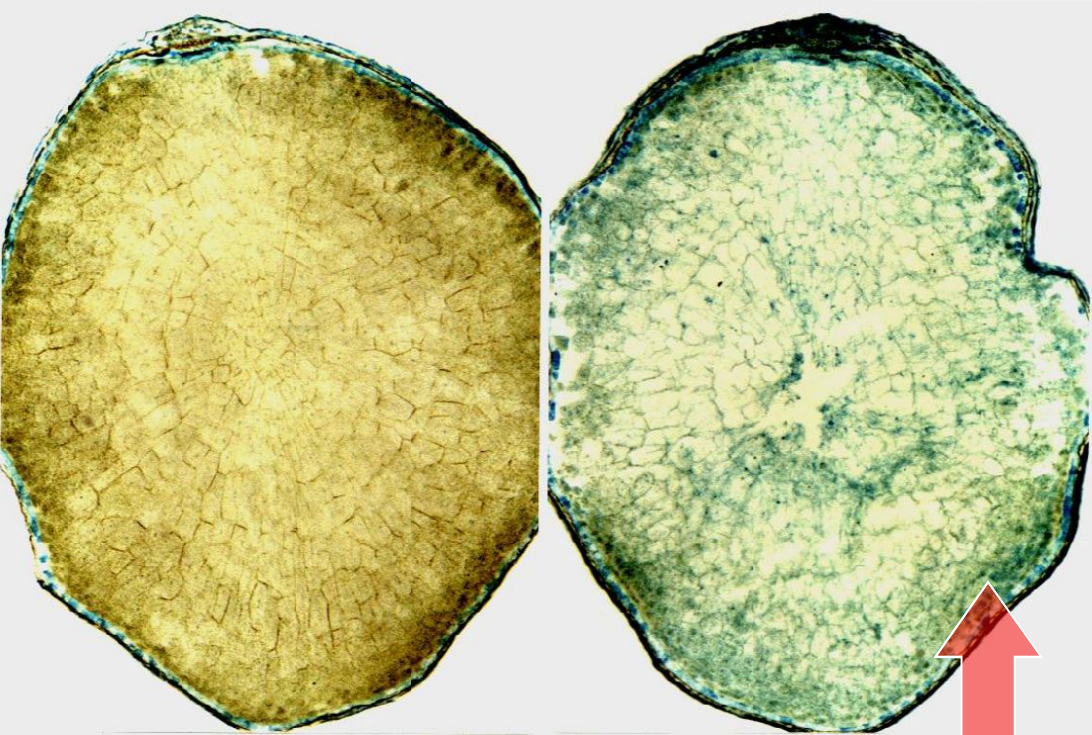
Genetic transformation



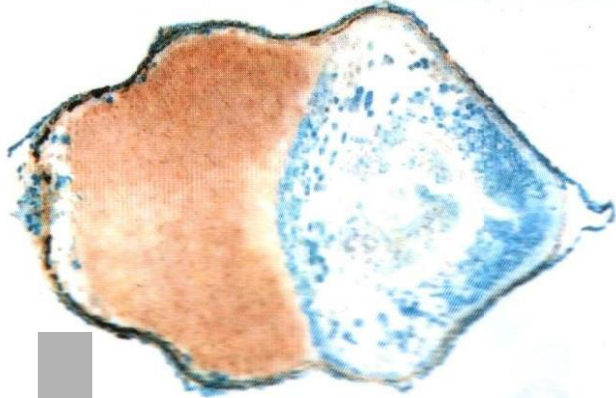
Results

Non-transformed

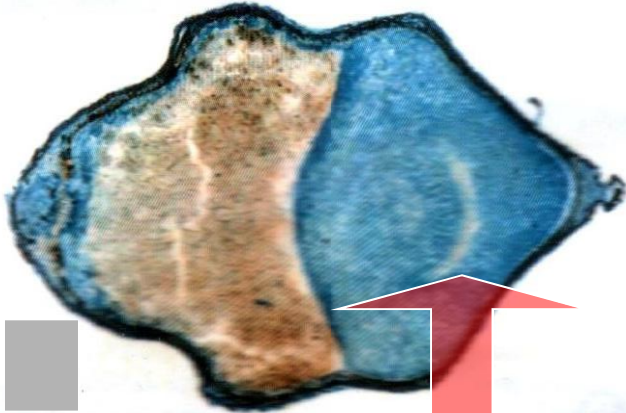
Transformed



Non-transformed



Transformed



Fe in blue

Results

- 3.7 X higher Fe and 1.4 X higher Zn, even after commercial polishing
- The Fe is bioavailable
- This material could prevent serious nutrient deficiencies



Mineral Biofortification in Rice: update

Table 1 | Summary of different approaches undertaken for mineral biofortification of rice.

Gene	Promoter	Cultivar	Fold increase in Fe/Zn	Reference
<i>AtNAS1+</i> , <i>Pvferritin+</i> , <i>Afphytase</i>	CaMV 35S, Glb-1, Glb-1	<i>Japonica</i> cv. Taipei 309	6.3/1.6 ¹	Wirth et al. (2009)
<i>HvNAS1</i>	Actin	<i>Japonica</i> cv. Tsukinohikari	3.4/2.3 ²	Masuda et al. (2009)
<i>HvNAS1</i> , <i>HvNAS1+HvNAA1</i> , <i>IDS3</i>	Genomic fragments	<i>Japonica</i> cv. Tsukinohikari	1.0/1.0 ² , 1.1/1.1, 1.4/1.3	Masuda et al. (2008)
<i>OsNAS1</i> , <i>OsNAS2</i> , <i>OsNAS3</i>	CaMV 35S	<i>Japonica</i> cv. Nipponbare	2.2/1.4, 4.2/2.2, 2.2/1.4	Johnson et al. (2011)
<i>OsNAS2</i>	Activation tagging	<i>Japonica</i> cv. Dongjin	3/2.7	Lee et al. (2011, 2012b)
<i>OsNAS3</i>	Activation tagging	<i>Japonica</i> cv. Dongjin	1/2.2	Lee et al. (2009b)
<i>OsNAS1</i>	GluB1	<i>Japonica</i> cv. Xiushui 110	1.0/1.3	Zheng et al. (2010)
<i>SoyferH-1</i>	Glu-B1	<i>Japonica</i> cv. Kitaake	3.0	Goto et al. (1999)
<i>PvFerritin+ rgMT</i>	Glb-1	<i>Japonica</i> cv. Taipei 309	2.0 ³	Lucca et al. (2001)
<i>SoyFer</i>	Glu-B1	<i>Indica</i> cv. IR68144	3.7/1.4	Vasconcelos et al. (2003)
<i>SoyFer</i>	Glu-B1; Glb-1	<i>Japonica</i> cv. Kitaake	3.0/1.1	Qu et al. (2005)
<i>OsFer2</i>	<i>OsGluA2</i>	Basmati rice (<i>Indica</i> cv. Pusa-Sugandh II)	2.1/1.4	Paul et al. (2012)
<i>TOM1</i>	CaMV 35S	<i>Japonica</i> cv. Tsukinohikari	1.2/1.6	Nozoye et al. (2011)
<i>OsYSL2</i>	<i>OsSUT1</i>	<i>Japonica</i> cv. Tsukinohikari	4.4	Ishimaru et al. (2010)
<i>OsIRT1</i>	Ubi	<i>Japonica</i> cv. Dongjin	1.1/1.1 ³	Lee and An (2009)
<i>OsYSL2+</i> , <i>SoyFerH2+</i> , <i>HvNAS1</i>	<i>OsSUT1</i> , Glb-1, Glb-1, Glu-B1, Act	<i>Japonica</i> cv. Tsukinohikari	6 or 4 ⁴ /1.6	Masuda et al. (2012)

Golden rice





“We all require Vit A, but children are the most susceptible to deficiency. RDA for 1-3 year olds is 300 μg per day. To give half this amount would keep healthy Vit A levels in blood circulation”

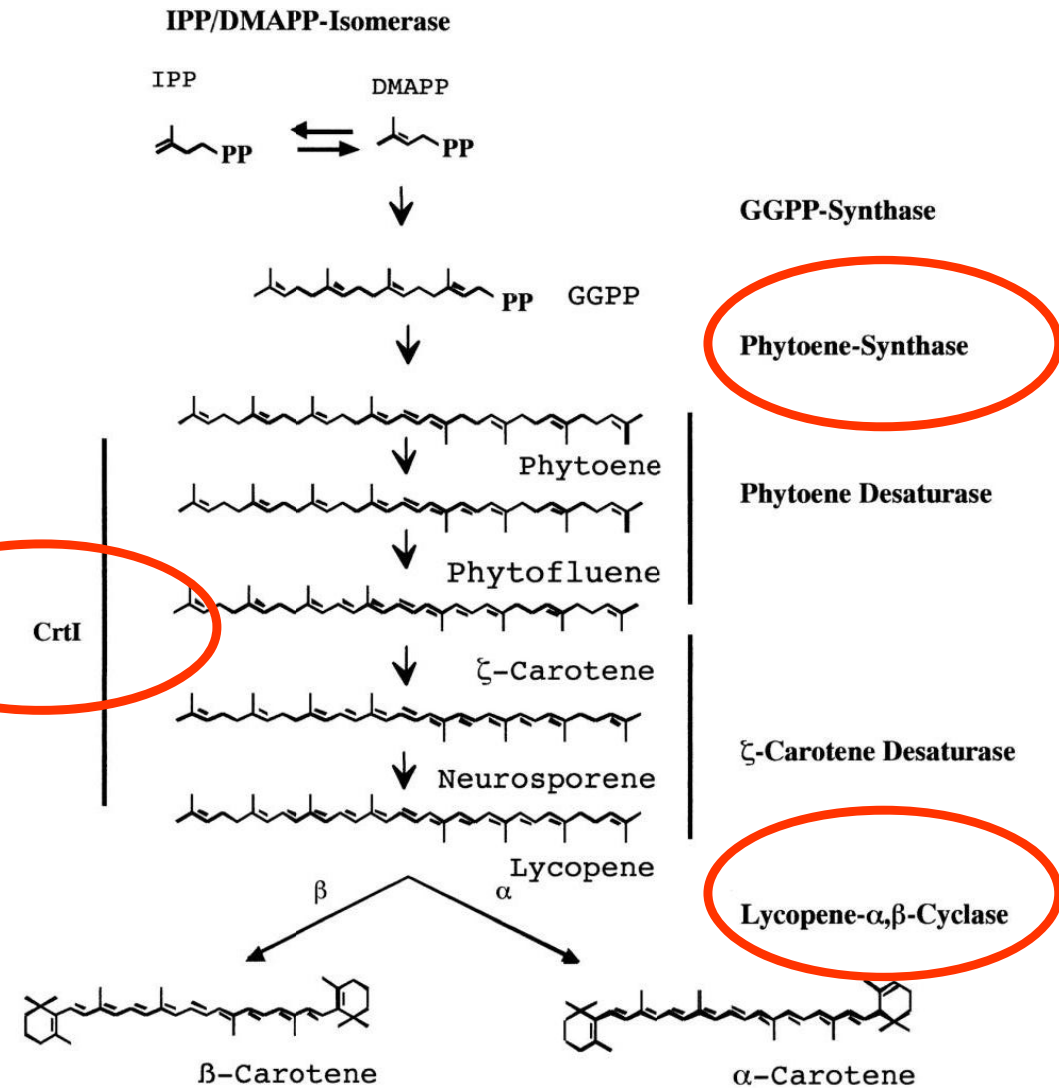
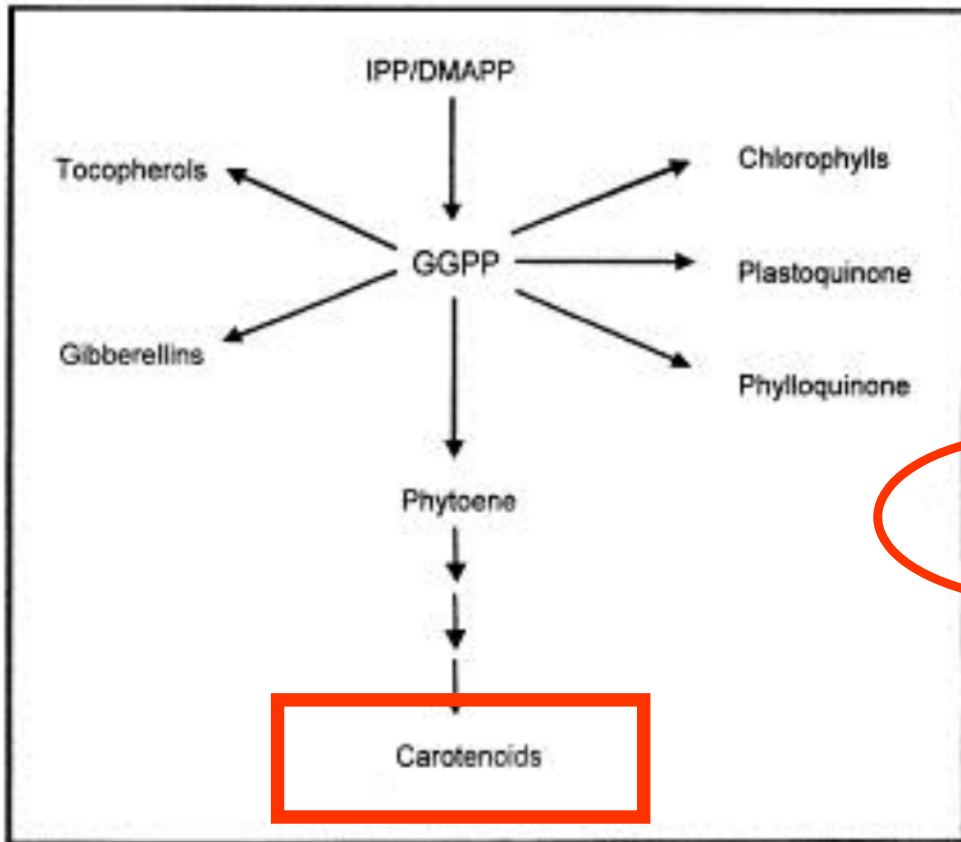


Rice

- Does not produce β -carotene (provitamin A)
- The genes are in the grain, but are “turned off”
- In societies of high rice consumption, lack of Vit A is a major health problem.



Strategy

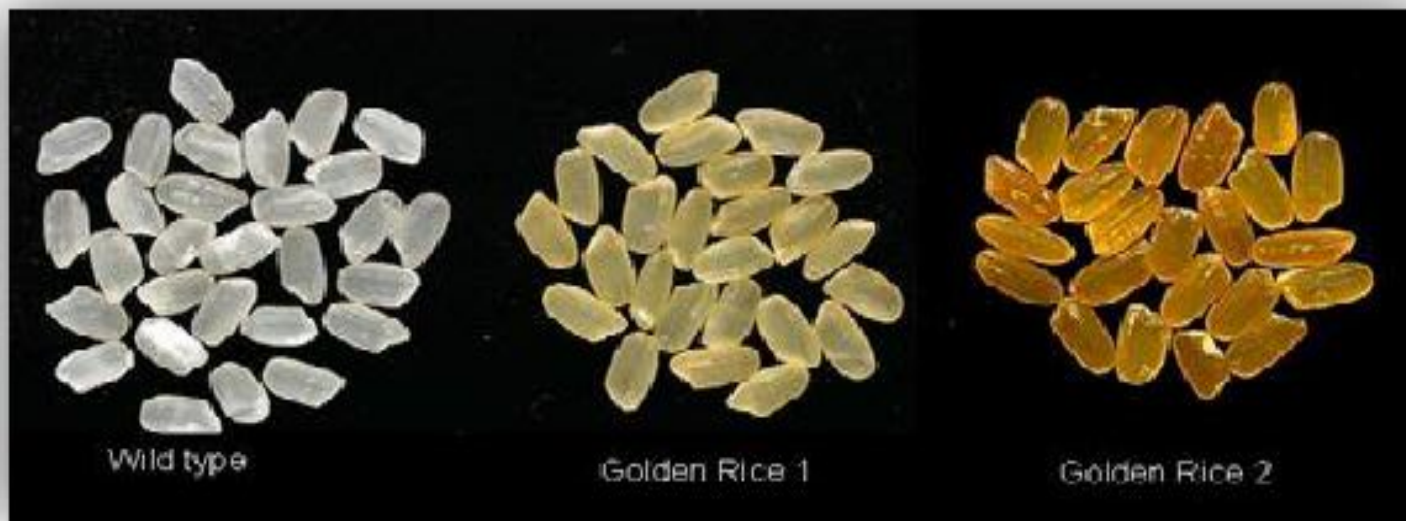
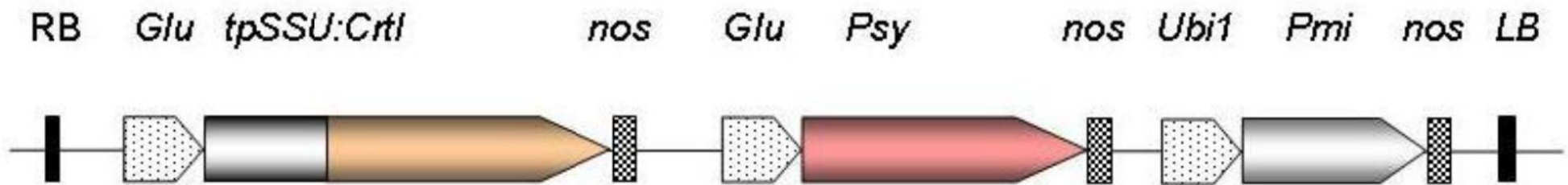


Golden rice 1

1.6 $\mu\text{g/g}$



Golden rice 2 37 $\mu\text{g/g}$



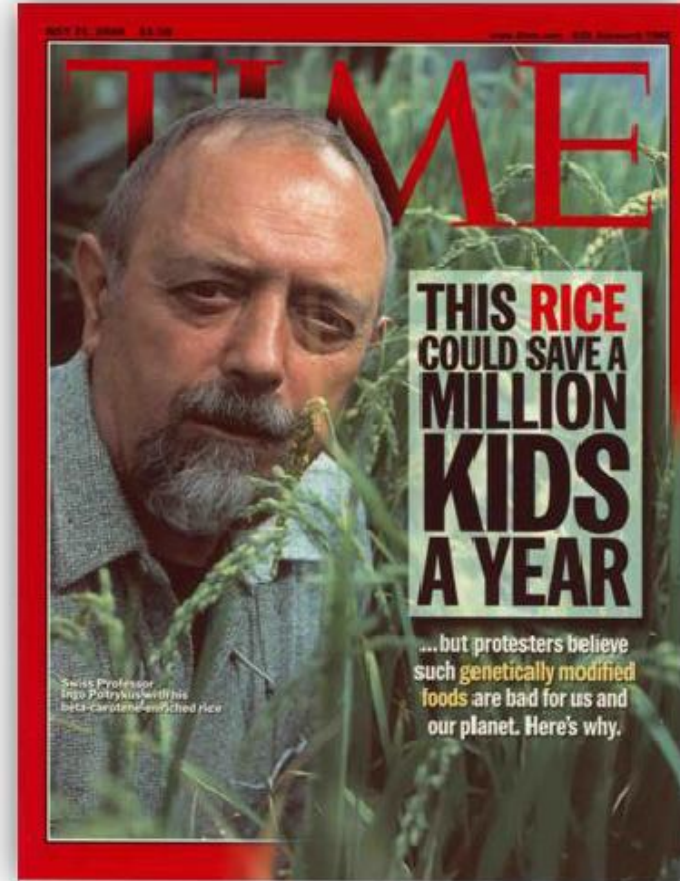


- ✓ **72 g provides half of the RDA! ***
- ✓ **Rice consumption levels in target countries (100-200 g of rice per child per day).**

Golden rice to be distributed freely without associated costs

* Based on retinol equivalency ratio for β -carotene of 12:1





Nutritional enhancement of transgenic soybean



Vasconcelos et al., (2006) Planta 1432-2048

*Vasconcelos, Clemente and Grusak (2014) Front. Plant Sci. 5:112. doi:
10.3389/fpls.2014.00112*

Food

- ✓ Grain
- ✓ Tofu
- ✓ Miso
- ✓ Flour
- ✓ Bread
- ✓ Candy
- ✓ Feed
- ✓ Cookies
- ✓ Pies
- ✓ Coffee
- ✓ Butter
- ✓ Milk
- ✓ Sprouts
- ✓ Oil
- ✓ (...)

Non-food

- ✓ Pencils
- ✓ Plastics
- ✓ Paints
- ✓ Fabrics
- ✓ Biodiesel
- ✓ Insecticides
- ✓ Paper
- ✓ Erasers
- ✓ Cosmetics
- ✓ Electric isolation
- ✓ Soaps
- ✓ Shampoos
- ✓ (...)

Soybean nutrition

- 14% Water
- 15% HC
- 15% Fiber
- 38% Protein
- 18% Lipids



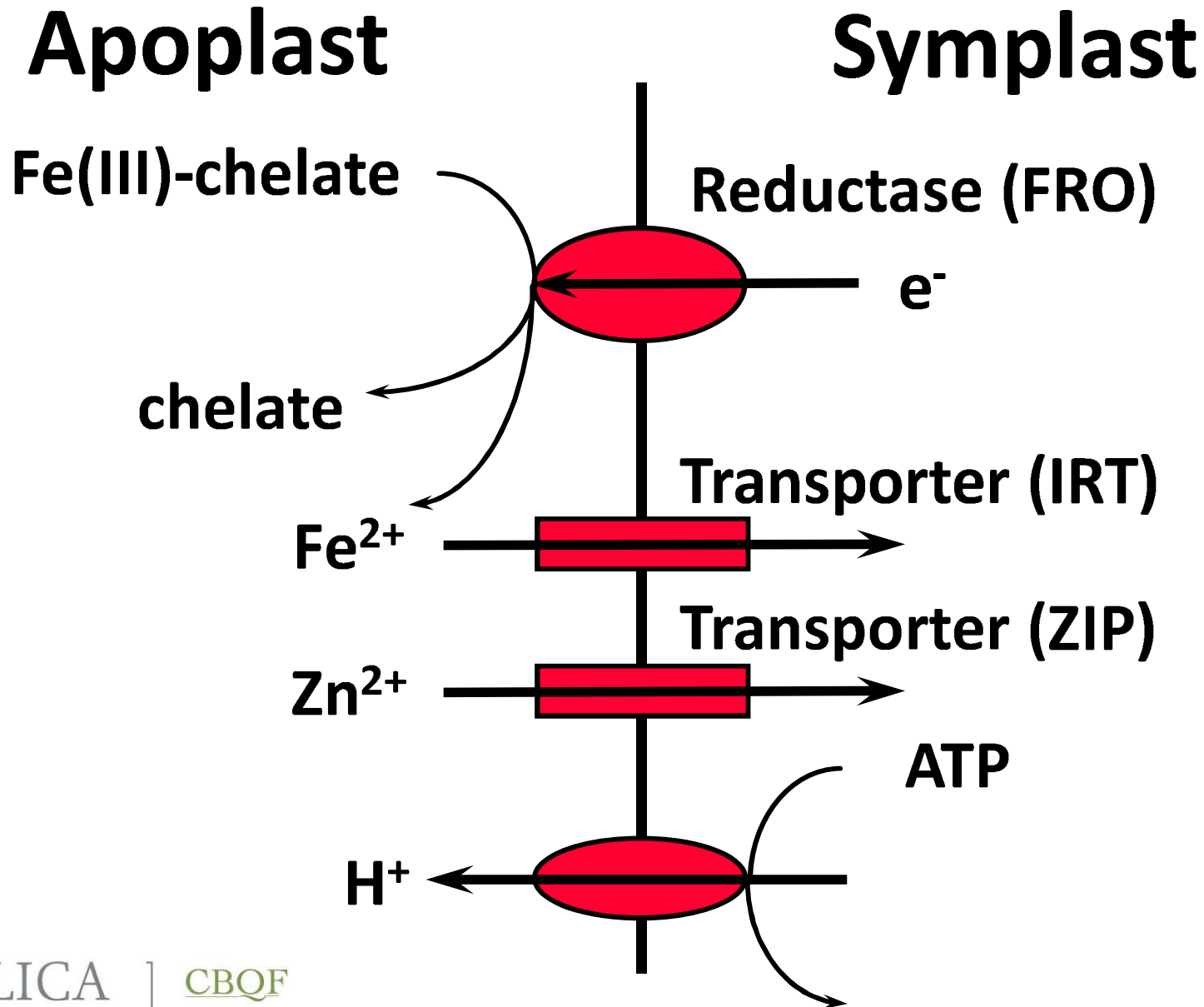
Good source of Fe, Ca, Zn, P, Mg, B, vitamins and folic acid

The problem...

1. Soybean is very susceptible to Fe deficiency (30% of world's arable land)
2. Low Fe absorption limits Fe that goes to the grain

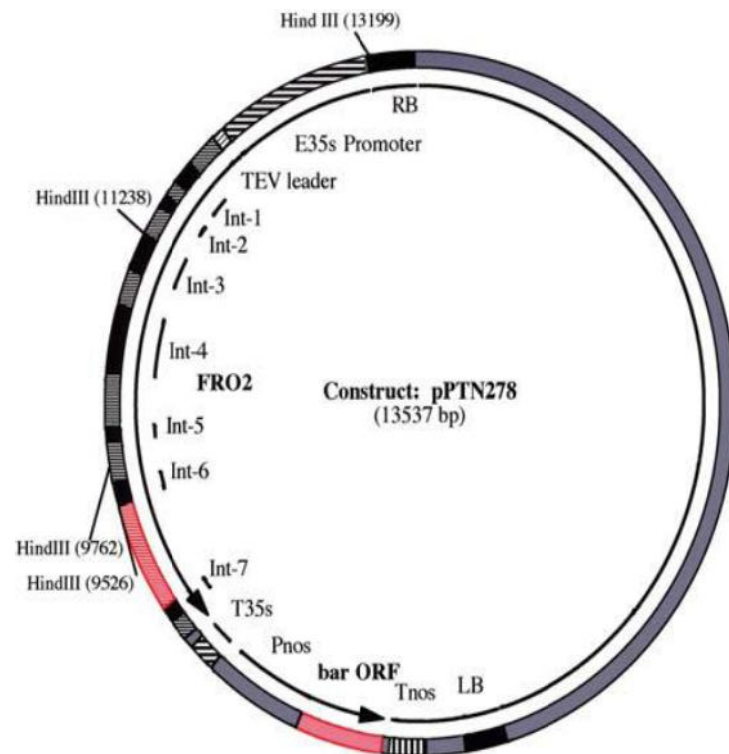


Strategy

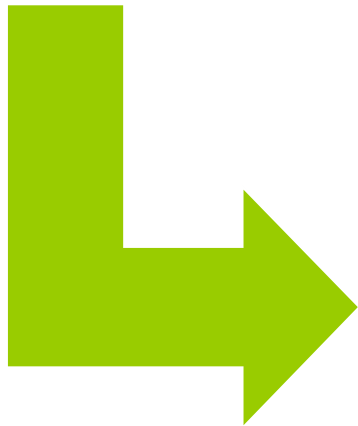


Materials

- *Agrobacterium* transformation with *AtFRO2*
- 38 transgenic lines with *AtFRO2* (2 parental lines)



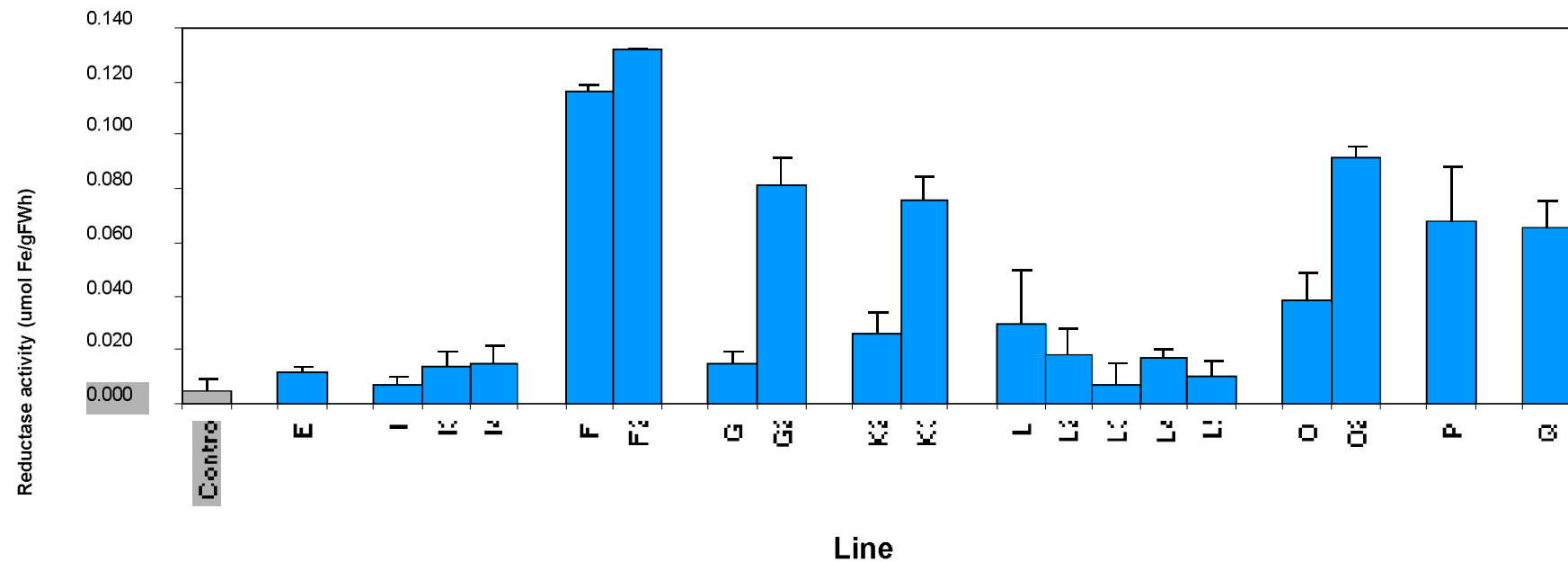
Soluble assay for reductase activity



Reductase:
$$\frac{\text{Av. Abs}_{535} \times \text{Vol. Sol}/1000}{0.02214 \times \text{Root FW} \times \text{Assay min}/60} = \mu\text{mol Fe/ g FW h}$$

Reductase activity ($\text{Fe}^{3+} \longrightarrow \text{Fe}^{2+}$)

23 x Higher activity!

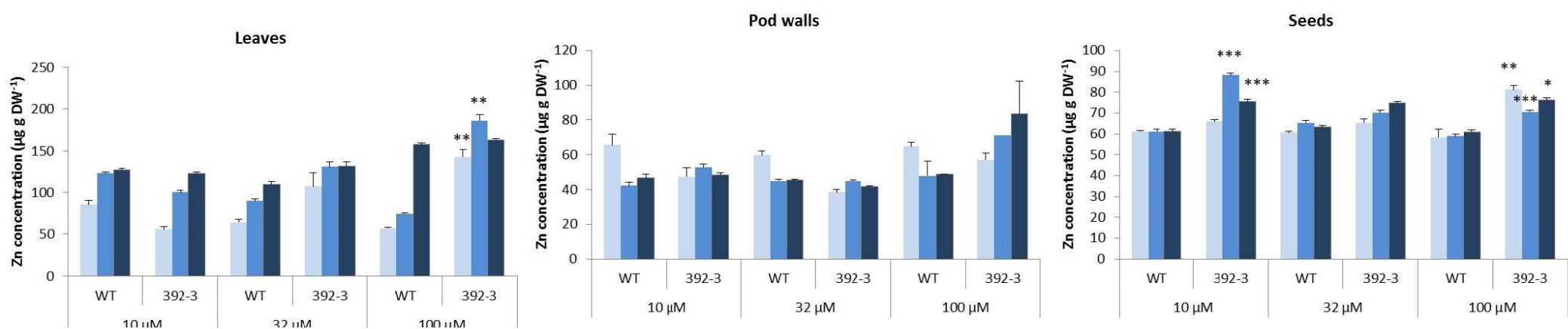
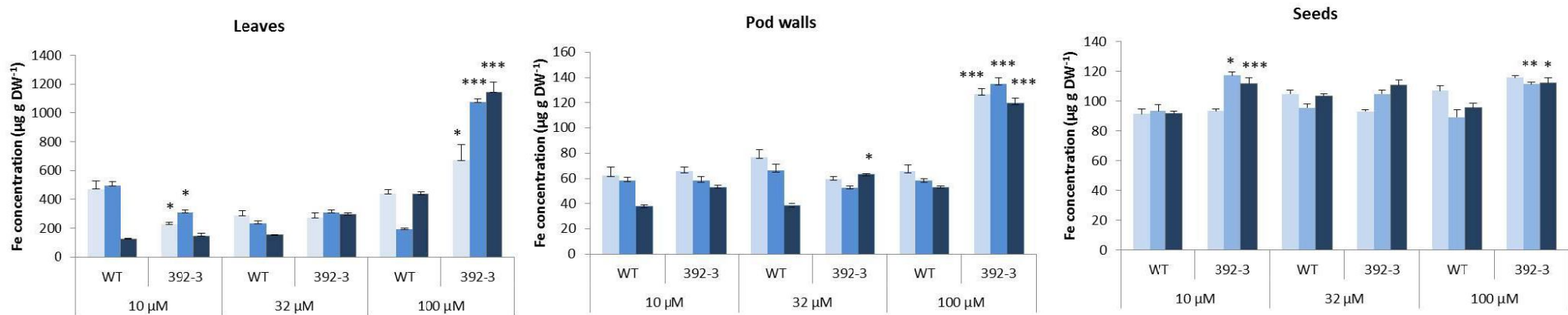




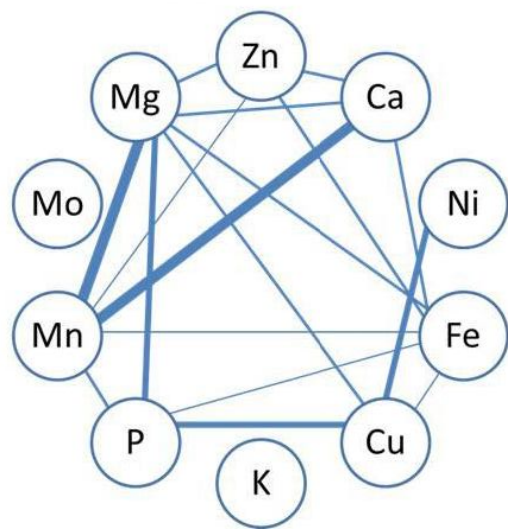
A- Control

B- 392-3

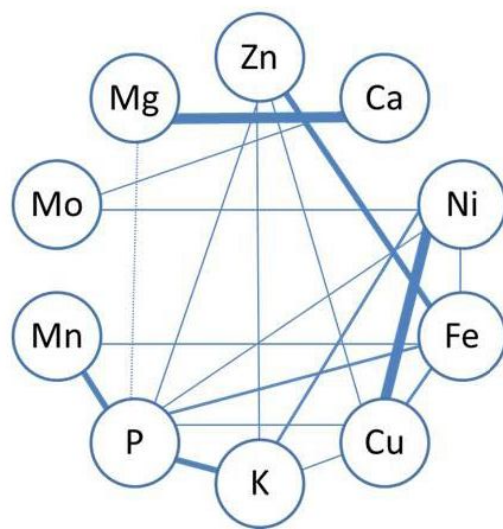
GF I GF II FM



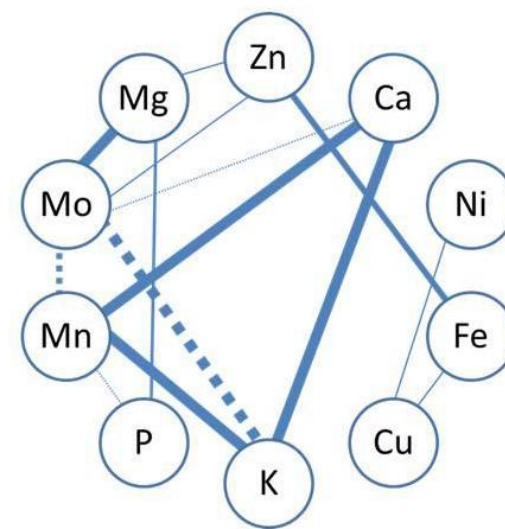
(a) Leaves



(b) Pod walls



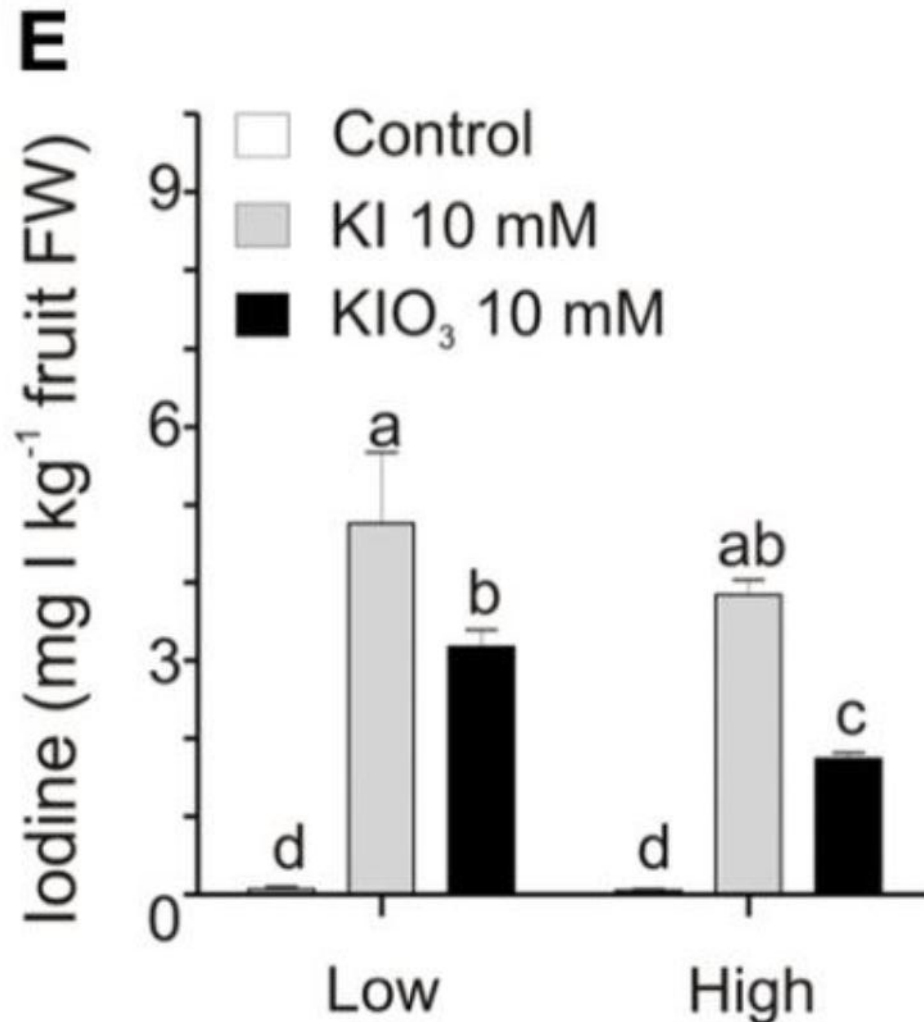
(c) Seeds



Other examples

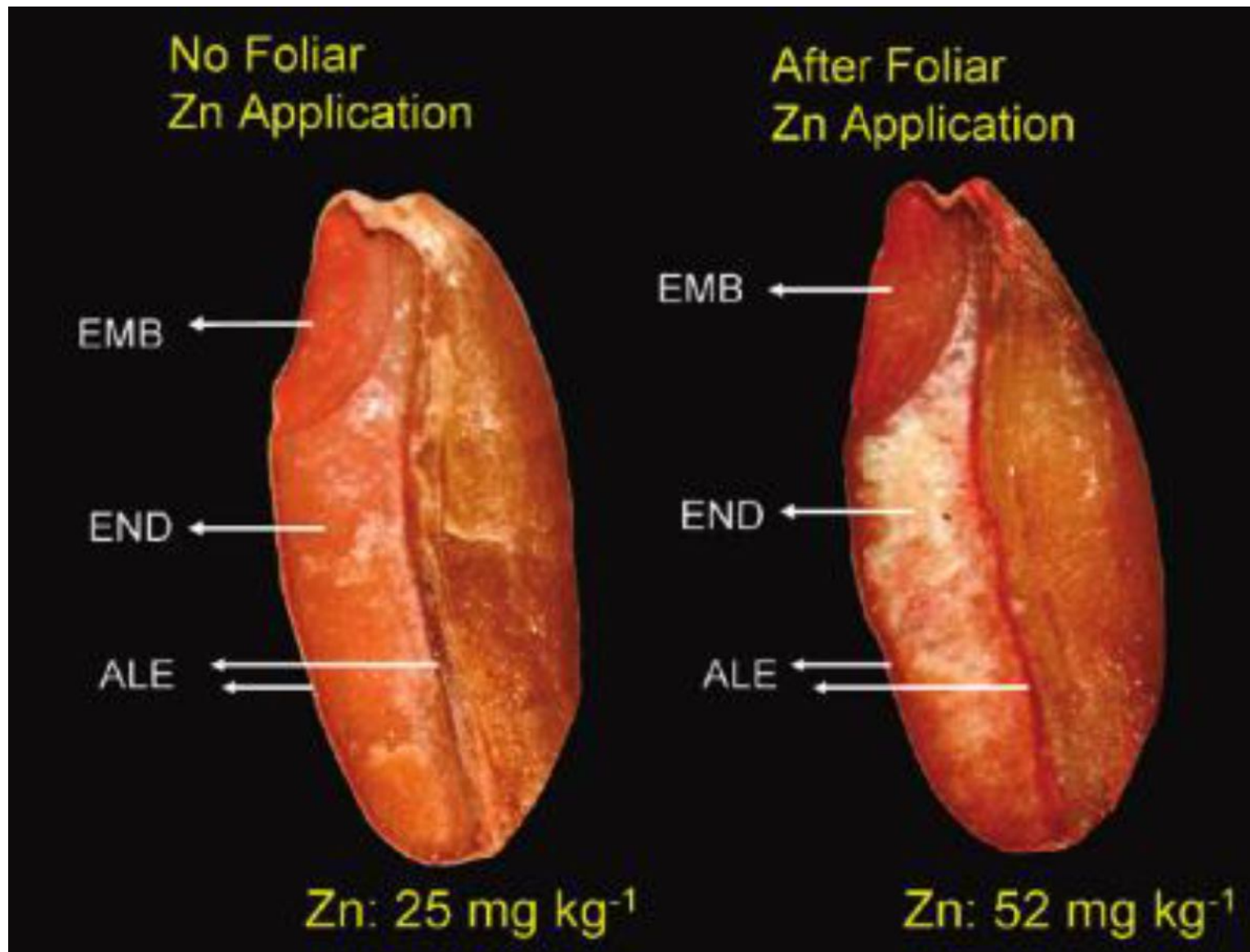
Tomato biofortified with iodine

(via soil fertilization)

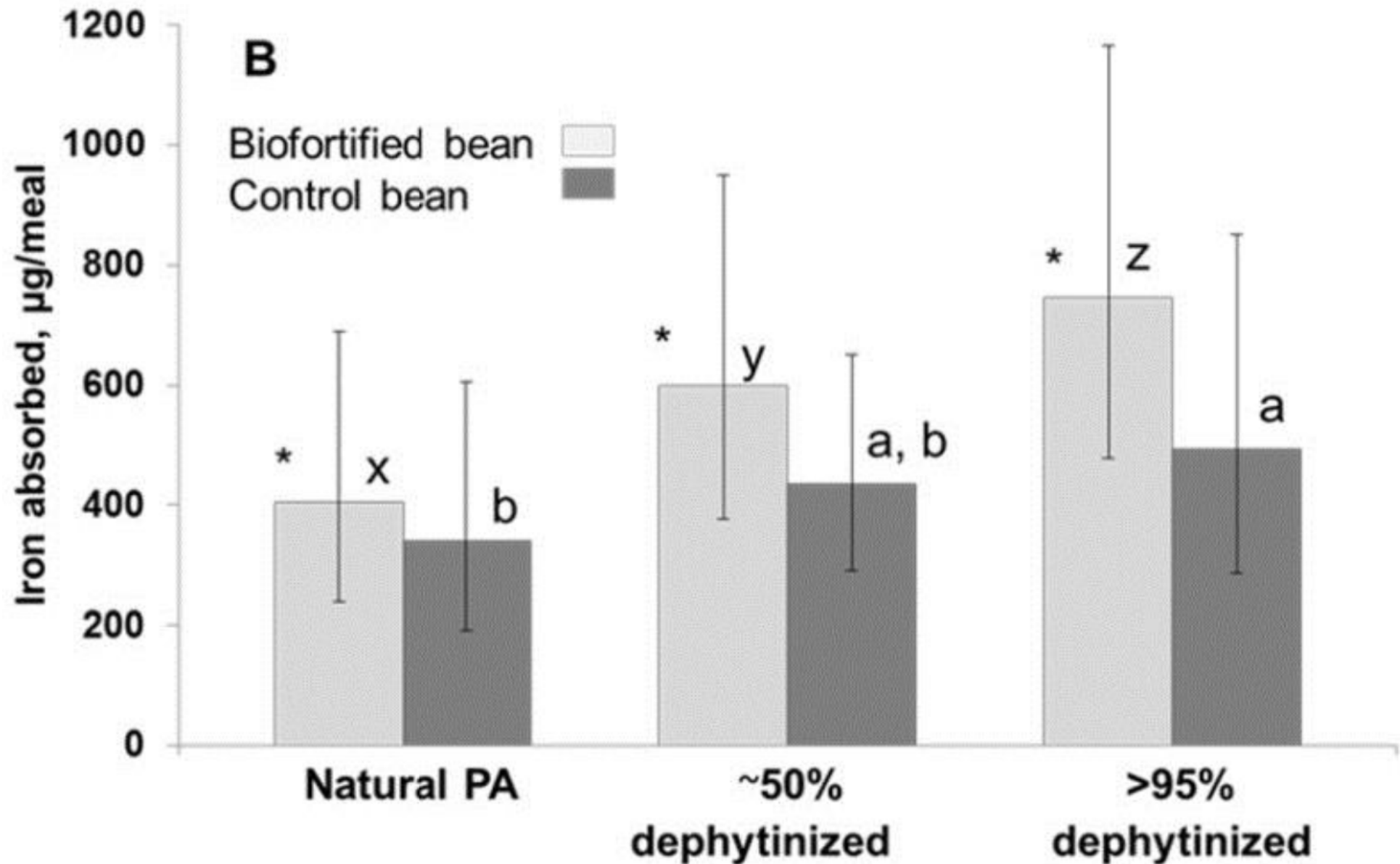


Wheat with Zinc

(via foliar application)



High iron bean (via breeding)



Biofortified crops in the field

- Iron Beans
 - Democratic Republic of Congo
 - Rwanda
- Iron Pearl Millet
- Vitamin A Cassava
- Vitamin A Maize
- Vitamin A Sweet Potato
- Zinc Rice
- Zinc Wheat

Target Crops, Nutrients, Countries, & Release Dates

Bean	Iron	DR Congo, Rwanda	2012
Cassava	Vitamin A	DR Congo, Nigeria	2011
Maize	Vitamin A	Nigeria, Zambia	2012
Pearl Millet	Iron	India	2012
Rice	Zinc	Bangladesh, India	2013
Sweet Potato	Vitamin A	Mozambique, Uganda	2007
Wheat	Zinc	India, Pakistan	2013

Biofortified crops in the news

March 09, 2015

“Zinc-Rich Maize Can Provide Adequate Zinc for Children, Study Finds”

Chomba et al., (2015) Journal of Nutrition



December 31, 2014

“Model Vitamin A Cassava Shop Launched in Ibadan, Nigeria”

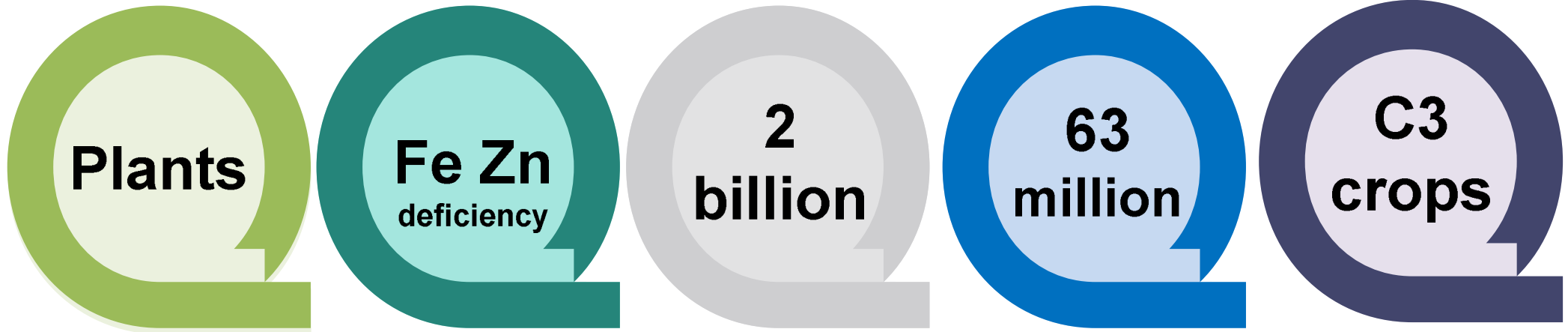


AfroPop, Rap and R&B Musicians Promote Healthier Diets—through Beans

<https://www.youtube.com/watch?v=fo6449Rd3I0>

Iron and Zinc

Why are they important



The basis of human food and nutrition.



Major global public health problem.



Suffer from Fe and Zn deficiency



Loss lives-year



95% of all plants are C3



Primary dietary source of Fe and Zn



Major protein sources



Impact of high CO₂

doi:10.1038/nature13179

Increasing CO₂ threatens human nutrition

Samuel S. Myers^{1,2}, Antonella Zanobetti¹, Itai Kloog³, Peter Huybers⁴, Andrew D. B. Leakey⁵, Arnold J. Bloom⁶, Eli Carlisle⁶, Lee H. Dietterich⁷, Glenn Fitzgerald⁸, Toshihiro Hasegawa⁹, N. Michele Holbrook¹⁰, Randall L. Nelson¹¹, Michael J. Ottman¹², Victor Raboy¹³, Hidemitsu Sakai⁹, Karla A. Sartor¹⁴, Joel Schwartz¹, Saman Seneweera¹⁵, Michael Tausz¹⁶ & Yasuhiro Usui⁹

Dietary deficiencies of zinc and iron are a substantial global public health problem. An estimated two billion people suffer these deficiencies¹, causing a loss of 63 million life-years annually^{2,3}. Most of these people depend on C₃ grains and legumes as their primary dietary source of zinc and iron. Here we report that C₃ grains and legumes have lower concentrations of zinc and iron when grown under field conditions at the elevated atmospheric CO₂ concentration predicted for the middle of this century. C₃ crops other than legumes also have lower concentrations of protein, whereas C₄ crops seem to be less affected. Differences between cultivars of a single crop suggest that breeding for decreased sensitivity to atmospheric CO₂ concentration could partly address these new challenges to global health.

experiments contribute more than tenfold more data regarding both the zinc and iron content of the edible portions of crops grown under FACE conditions than is currently available in the literature. Consistent with earlier meta-analyses of other aspects of plant function under FACE conditions^{14,15}, we considered the response comparisons observed from different species, cultivars and stress treatments and from different years to be independent. The natural logarithm of the mean response ratio ($r = \text{response in elevated } [\text{CO}_2] / \text{response in ambient } [\text{CO}_2]$) was used as the metric for all analyses. Meta-analysis was used to estimate the overall effect of elevated [CO₂] on the concentration of each nutrient in a particular crop and to determine the significance of this effect (see Methods).

We found that elevated [CO₂] was associated with significant decreases in the concentrations of zinc and iron in all C₃ grasses and le-

! C3 plants will have lower **Fe, Zn, protein** in edible plant part

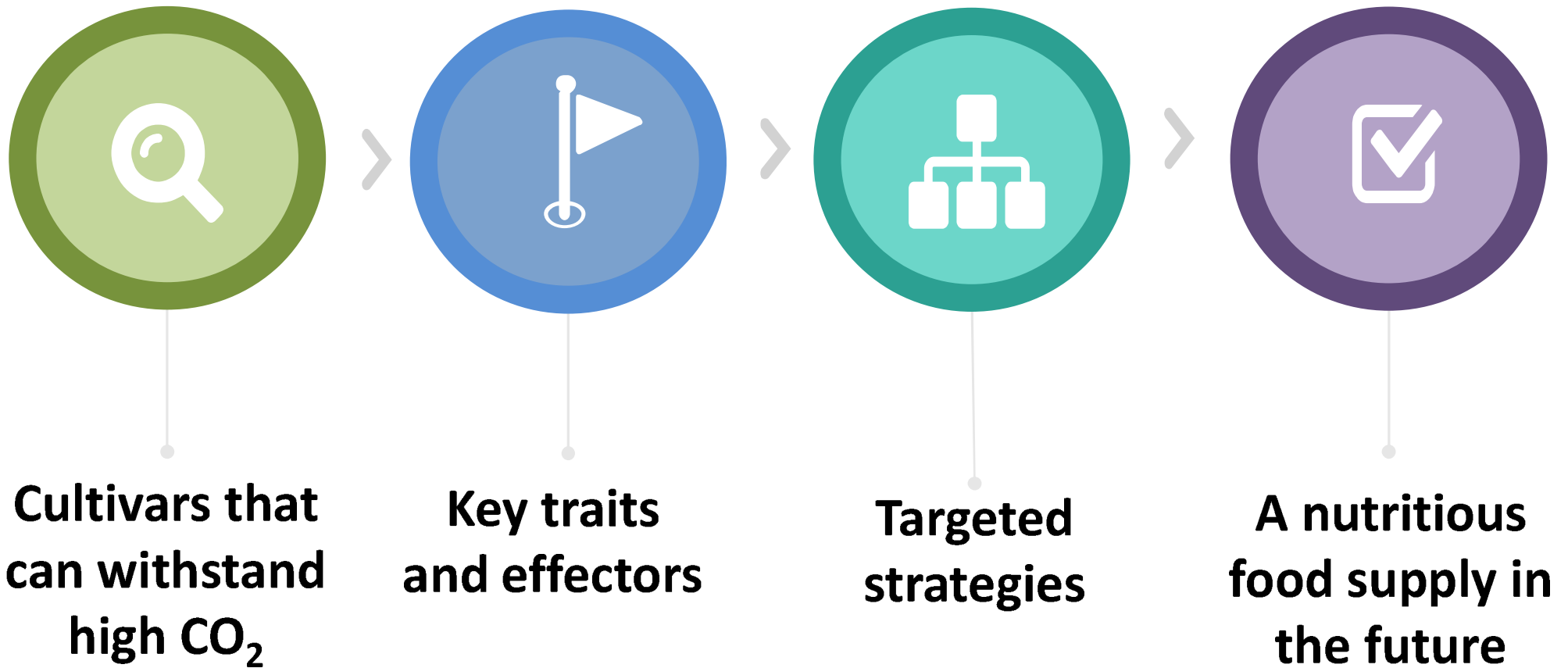


Global challenge

Maintain a high nutritional
status

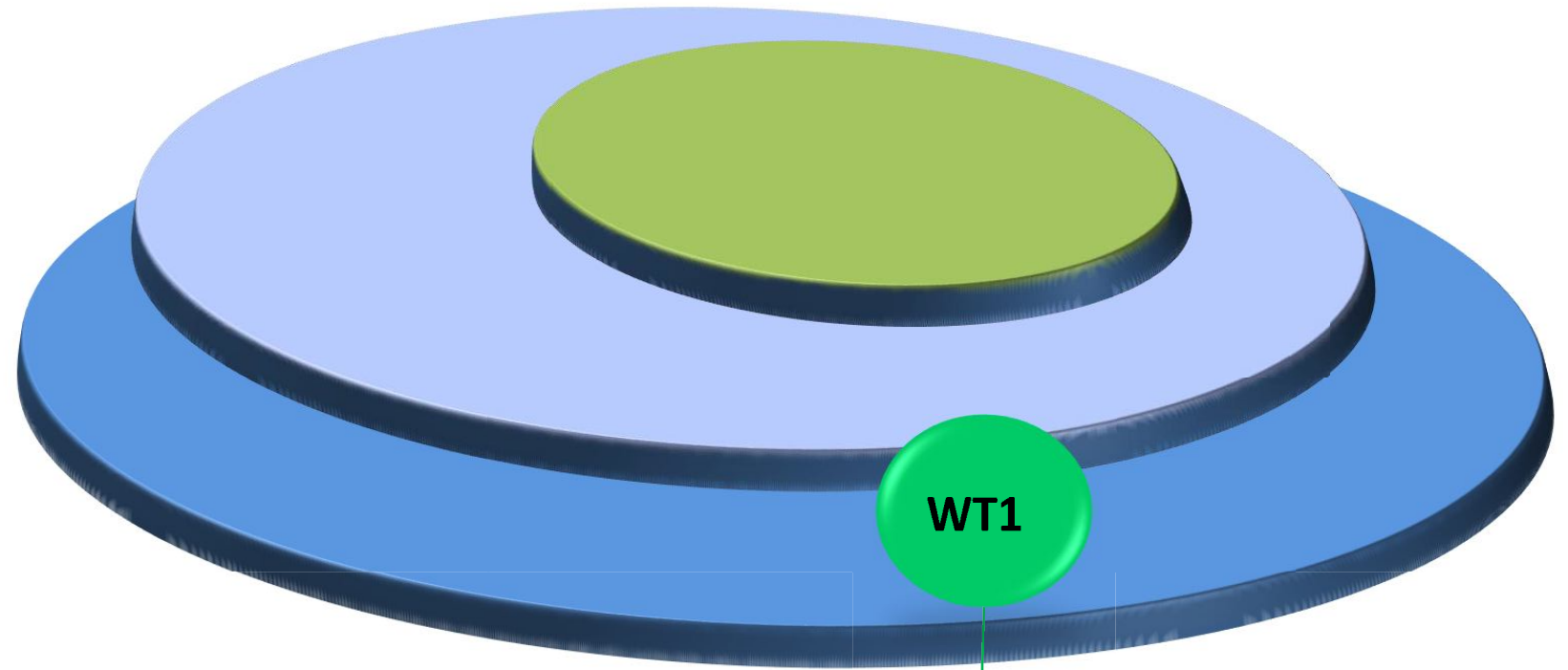
How to address this global challenge?

The necessary steps



New FCT project (starts May 1st)!

NUTRI4CAST

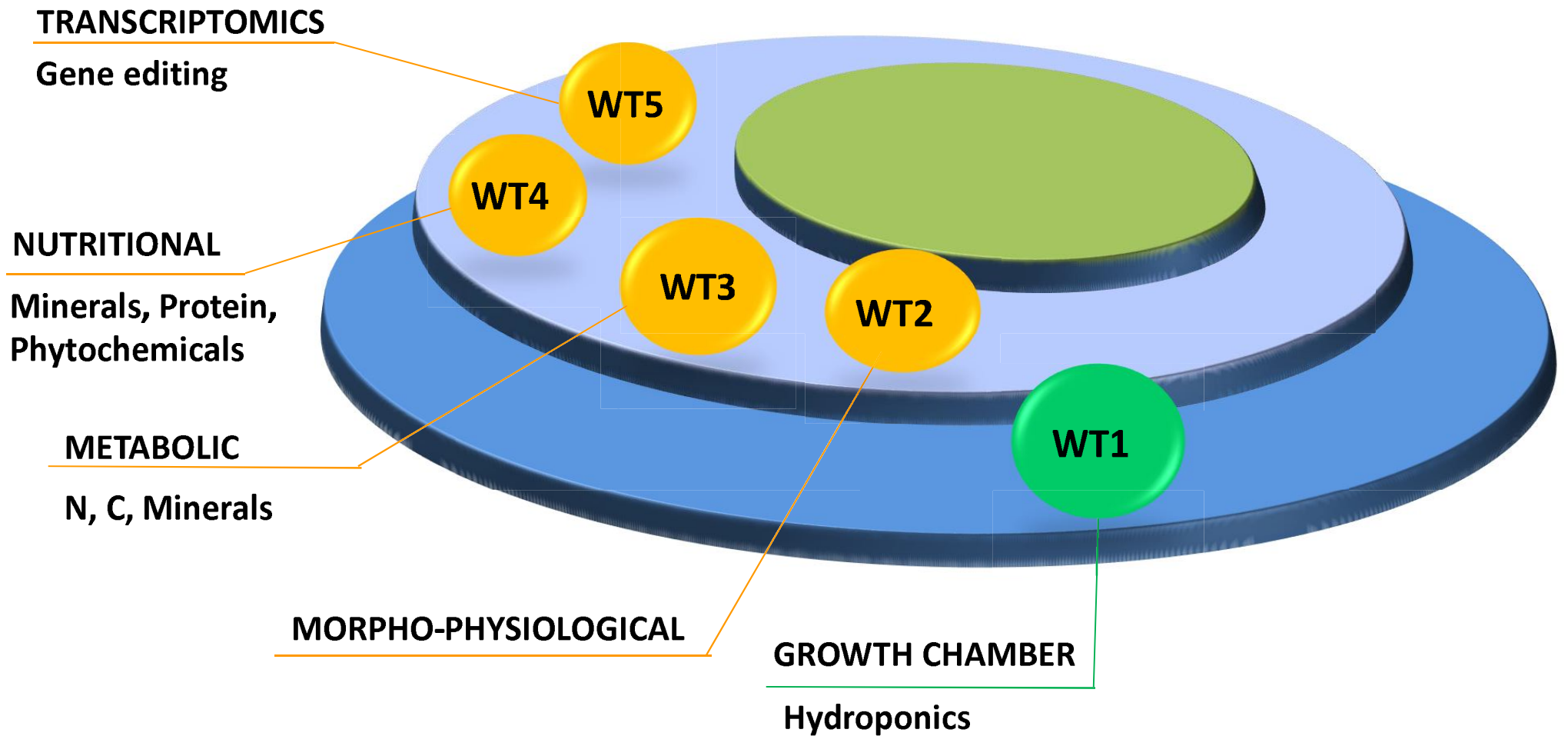


GROWTH CHAMBER

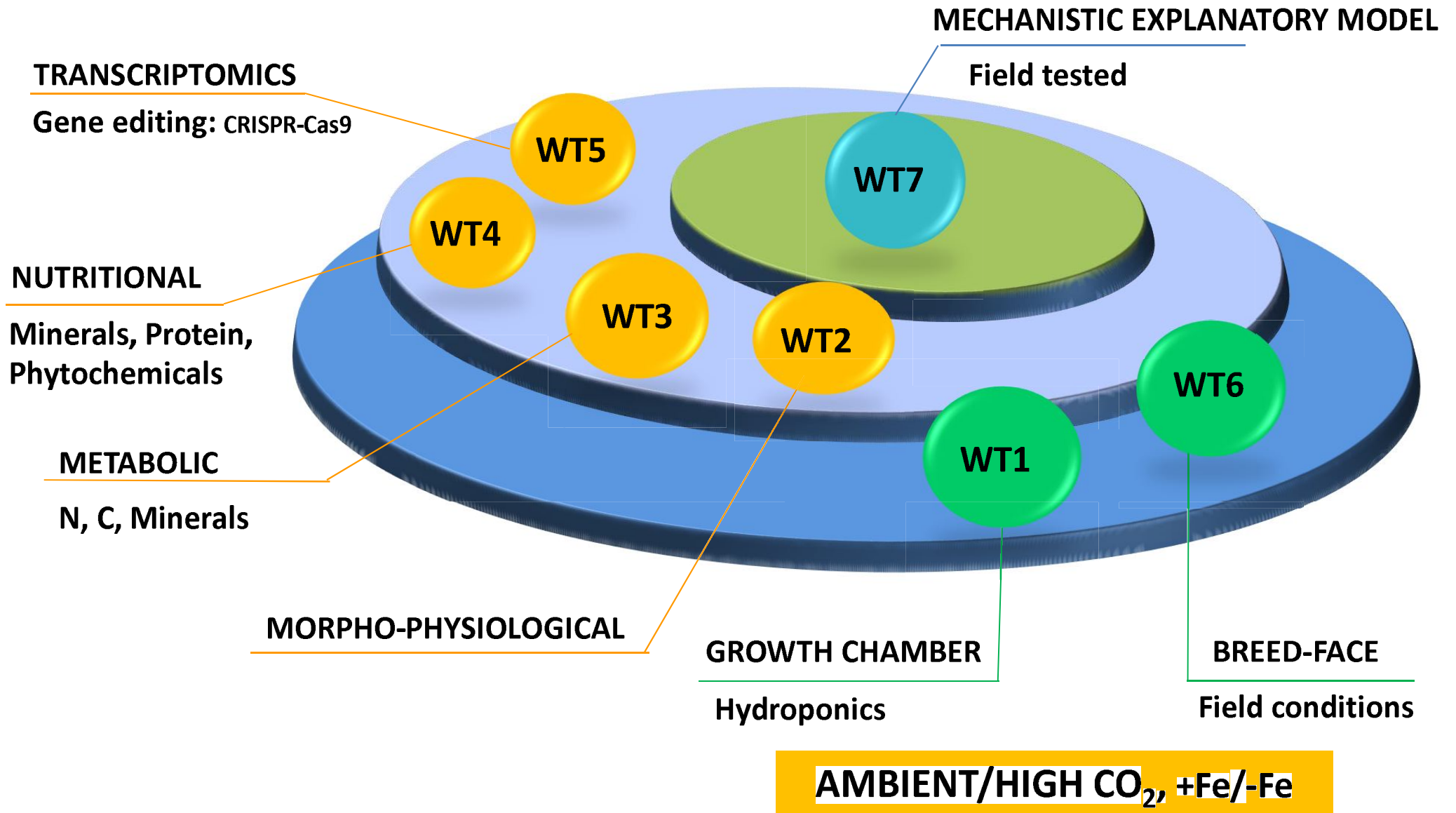
Hydroponics

AMBIENT/HIGH CO₂, +Fe/-Fe

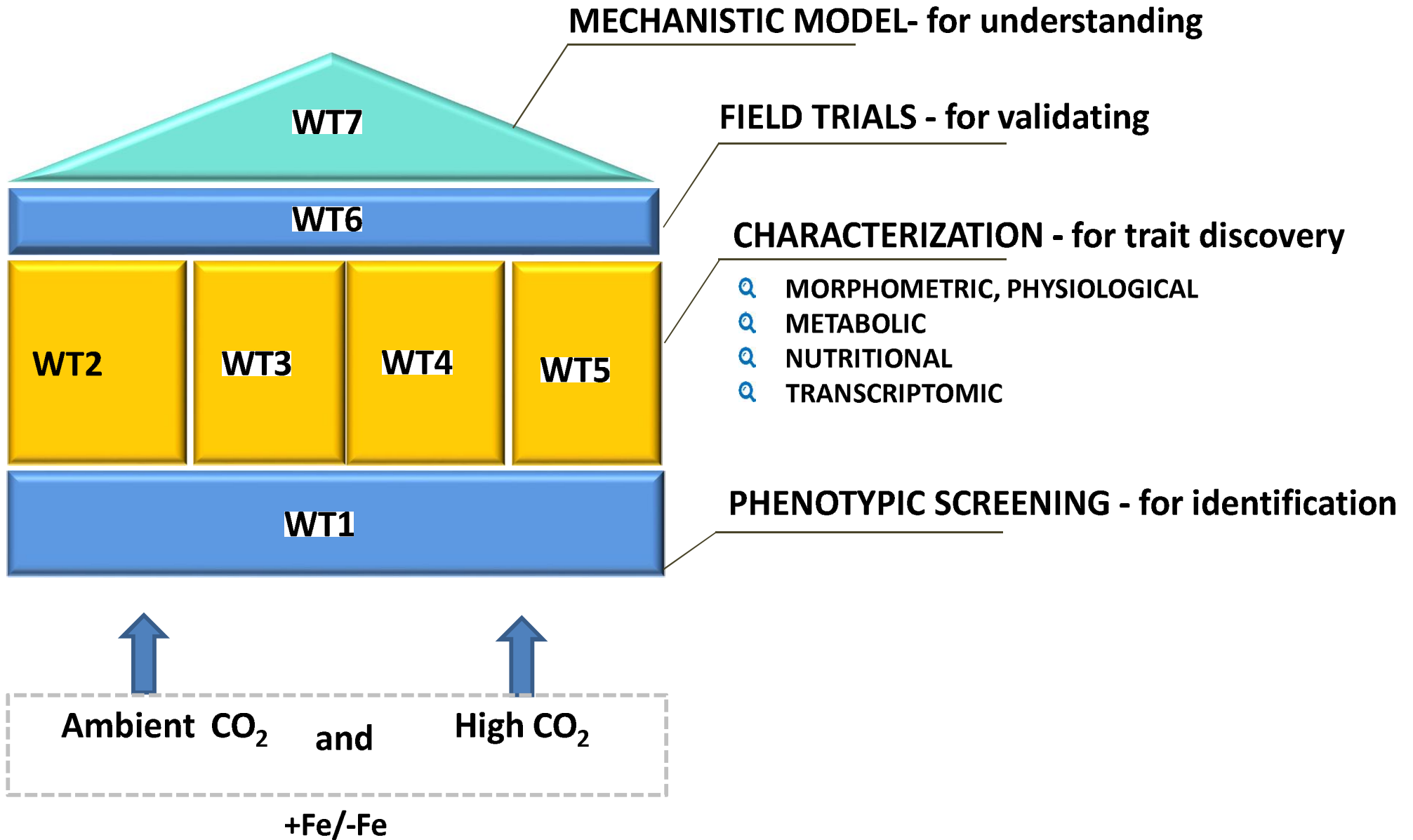
NUTRI4CAST



CONTROL-N



CONTROL-N



High Throughput Phenotyping

Innovation

Partner

When?

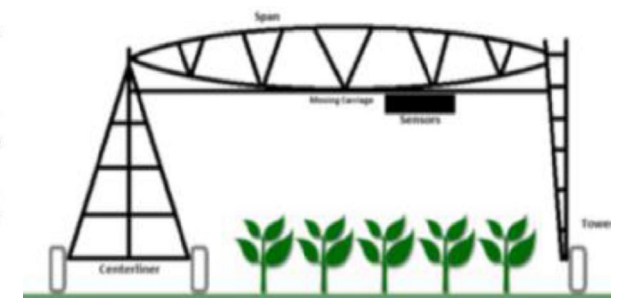
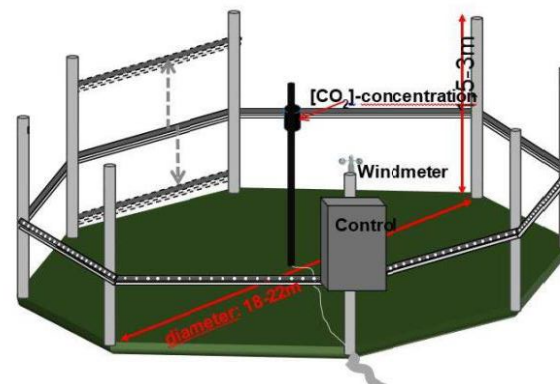
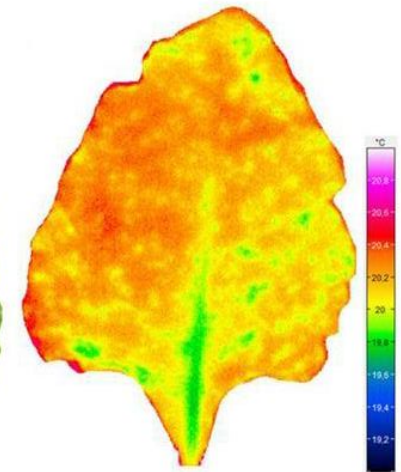
What?

WT2

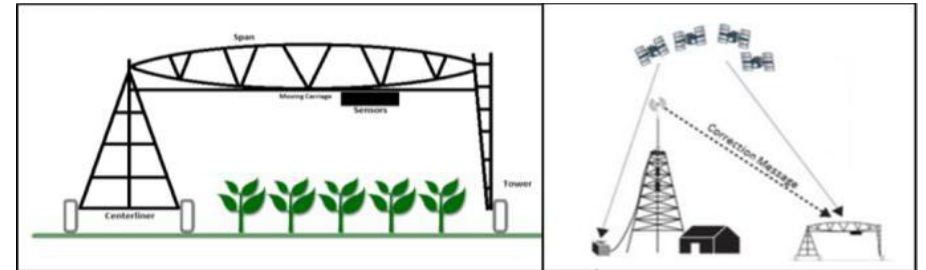
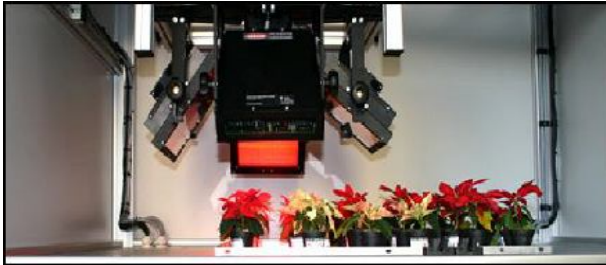
WT3



WT7



Milestones



Digital phenotyping

Nutritional Metabolic analysis

Transcriptomic analysis

Breed-FACE Trial

Morphometric Physiological analysis

Screening under controlled CO₂

Predictive Model

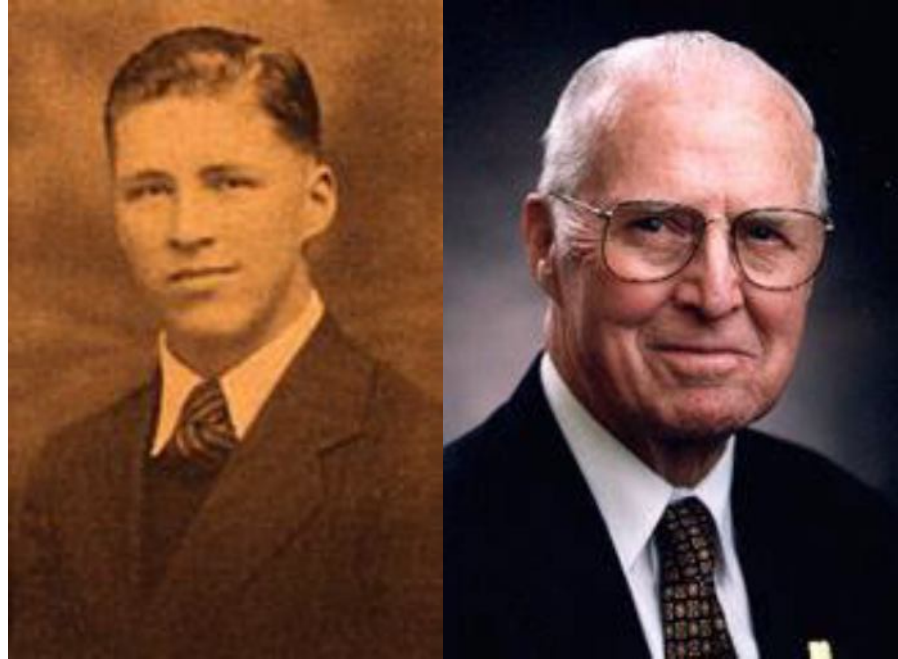
Year 1

Year 2

Year 3

Norman Borlaug

1914–2009



“Civilization as it is known today could not have evolved, nor can it survive, without an adequate food supply “

Thank you for your attention!

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