Biotechnology, biofortification and healthy diets: food systems interventions for enhanced nutrition
&
The case of folates

Dominique Van Der Straeten

Laboratory of Functional Plant Biology

Ghent University – Campus Ledeganck

Plant B+B Café, May 20, 2021

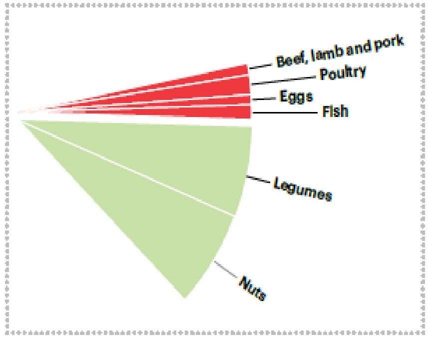
Outline

- Intro on Food systems interventions for enhanced nutrition:
  - -healthy diets anno 2021
  - -why biofortification
  - -why biotech for biofortification: the potential of metabolic engineering
  - -where do we stand: current realizations
- Case study: folates, vitB9
- Roadmap to SDG2

What is a healthy diet in the 21st century?

The ‘planetary health plate’
Target: 2500 kcal/day

The EAT Lancet Commission Report, 2019



The food system as a health-planetary nexus

Transformation to healthy diets by 2050 will require substantial dietary shifts. Global consumption of fruits, vegetables, nuts and legumes will have to double, and consumption of foods such as red meat and sugar will have to be reduced by more than half. A diet rich in plant-based foods and with fewer animal source foods confers both improved health and environmental benefits.

Walter Willett, Harvard University

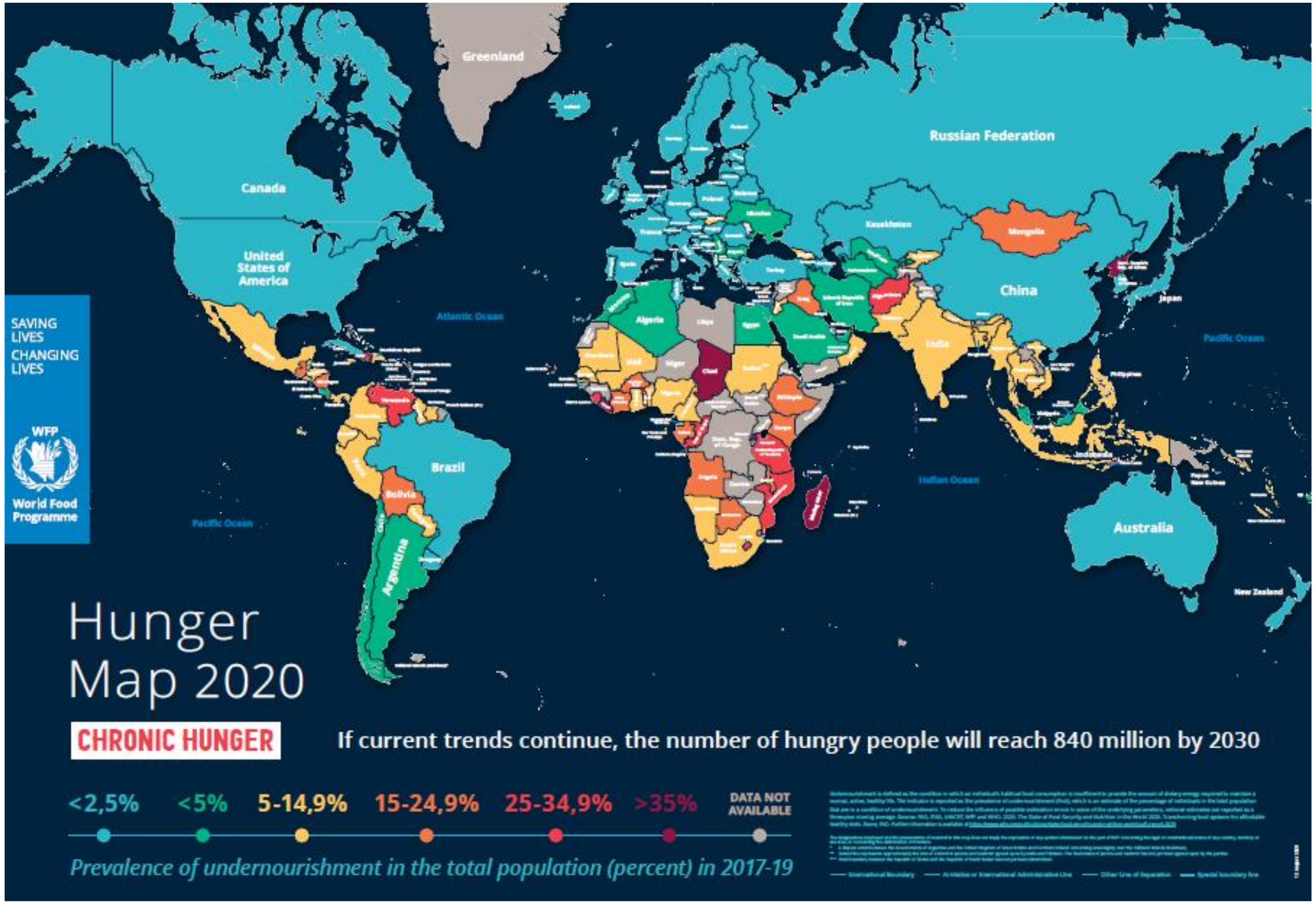
The EAT Lancet Commission Report, 2019

Global hunger map, 2020

HUNGER:

- ***Undernourishment:***
Lack of calories (acute/chronic)

- ***Malnutrition:***
Lack of essential nutrients Proteins or micronutrients



Food insecurity:

- ***Population explosion***
(7,8 billion anno 2021 8,5 billion by 2030)

- ***Linked to conflict,***
global peace is essential

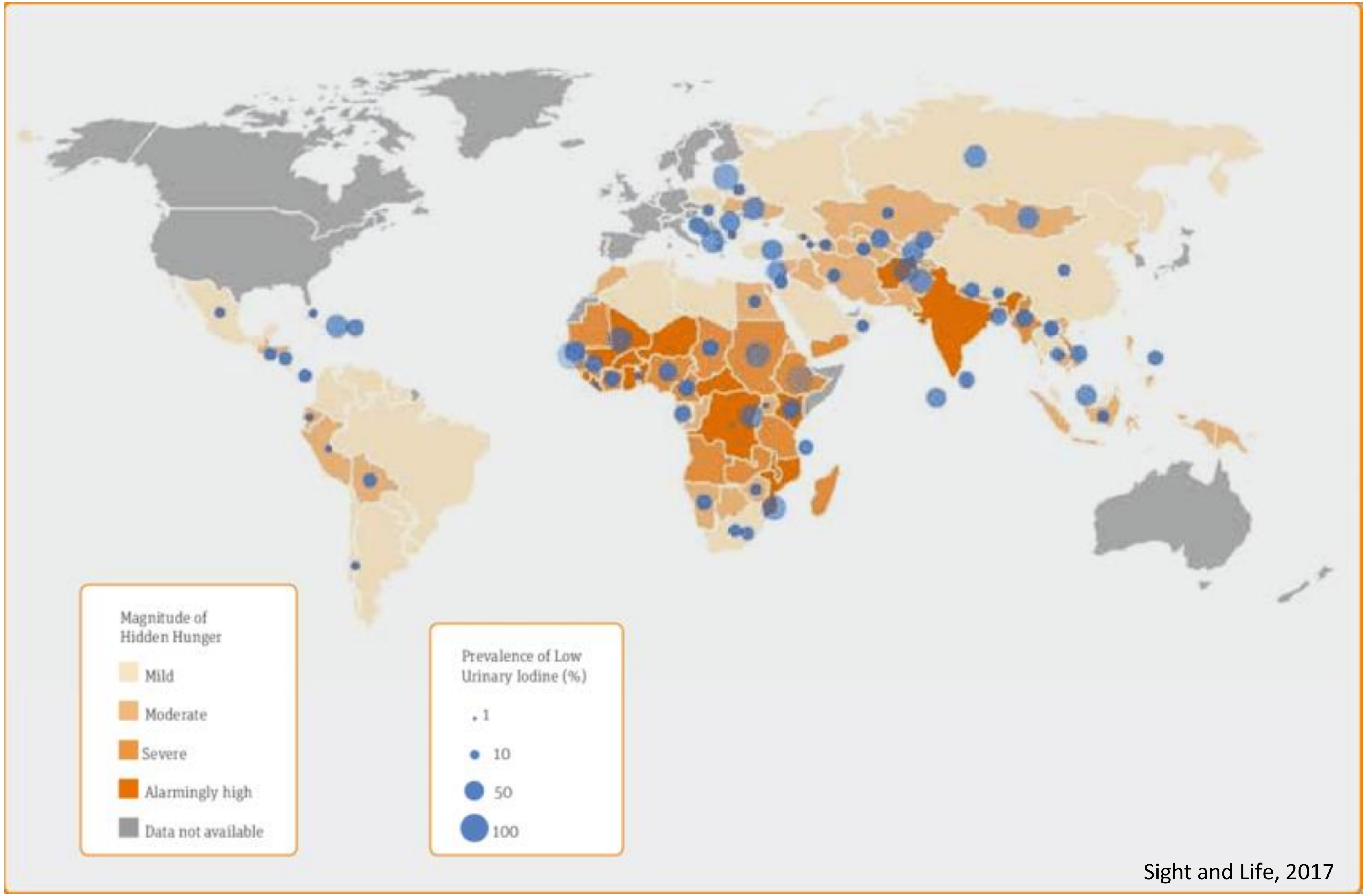
- ***Climate change** effects*

<u>Education</u>** of the poor **is key

Hidden hunger map, 2017

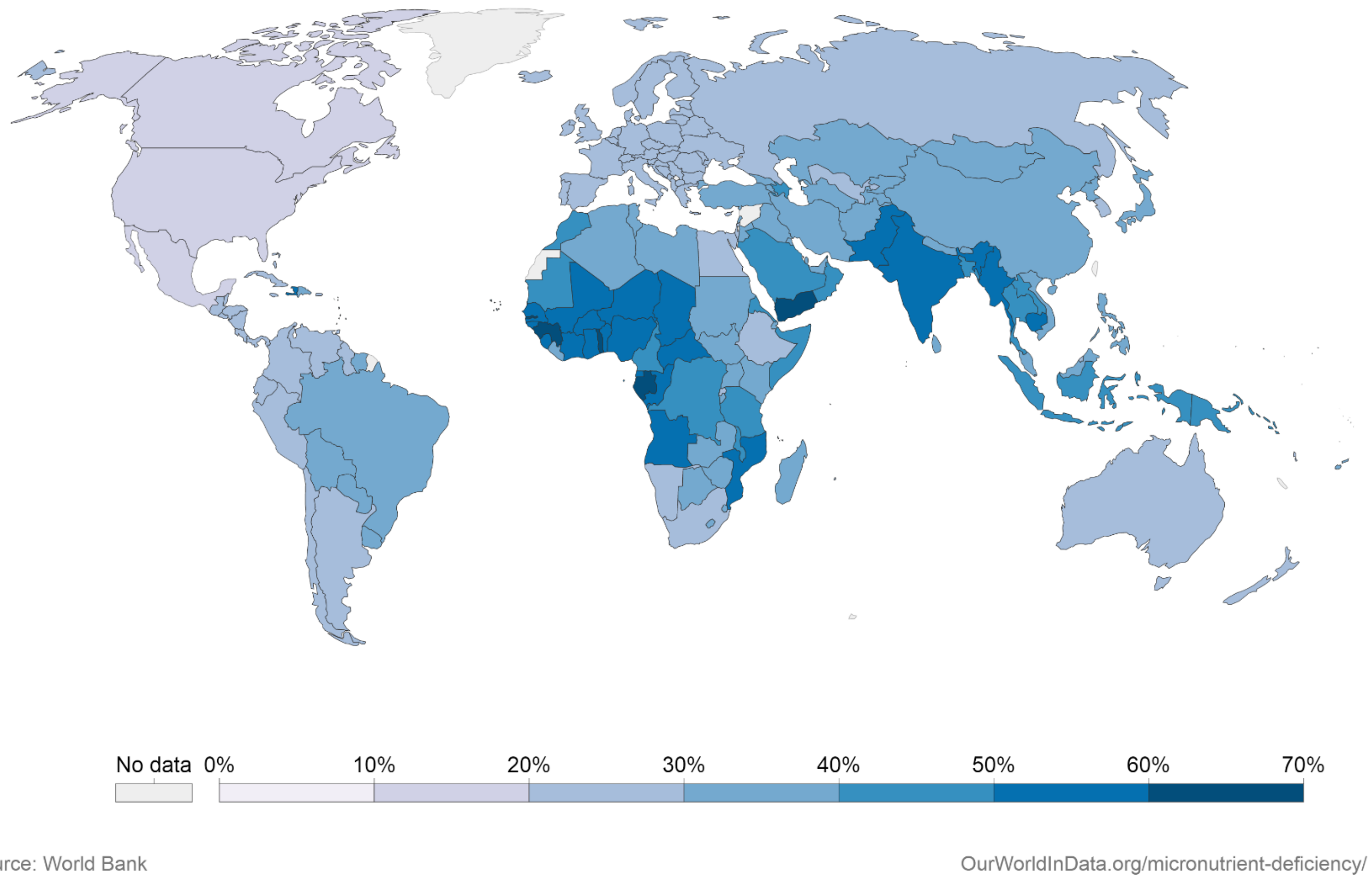
Vitamin & mineral Deficiencies

Important in immune response!!



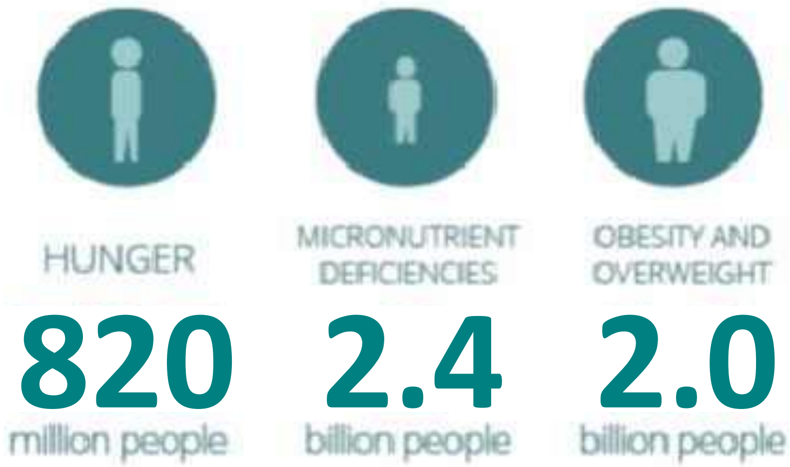
Sight and Life, 2017

Prevalence of anemia in pregnant women



Globally, anemia impacts about **40% of pregnant women** and **more than 20% of non-pregnant women**
Iron deficiency + folate deficiency related

UN-SDG2:
Zero hunger by 2030?



UN, 2015; IPES, 2016; Lancet, 2016

Hunger vs. 'hidden hunger' (MNM)

Fighting micronutrient deficiencies

- Industrial fortification of food

  > specialized infrastructure

- Supplementation with pills

  > problem of reaching target population or not taken on regular basis

- Diet diversification

  > change of dietary habits, availability, accessibility, affordability > *Education*

- *Biofortification*

  > Valuable complementary approach

Blancquaert *et al.*, *JXB*, 2014

Hippocrates, 460 BC: "*Let food be your medicine and medicine your food*"
Quality food is a prerequisite for health and well-being!

Requirements for successful biofortification

Reaching a **measurable, significant impact** on nutritional status:

1. **Higher** nutrient **density** in low nutrient crop products
2. **Efficacy**:

   - retention of desired levels after storage (*stability*)/processing /cooking

   - bioavailability upon food consumption

3. **Acceptance** to grow + willingness to buy and eat biofortified varieties

Van Der Straeten *et al.*, *NComm*, 2020

Three approaches to biofortification

Biofortification

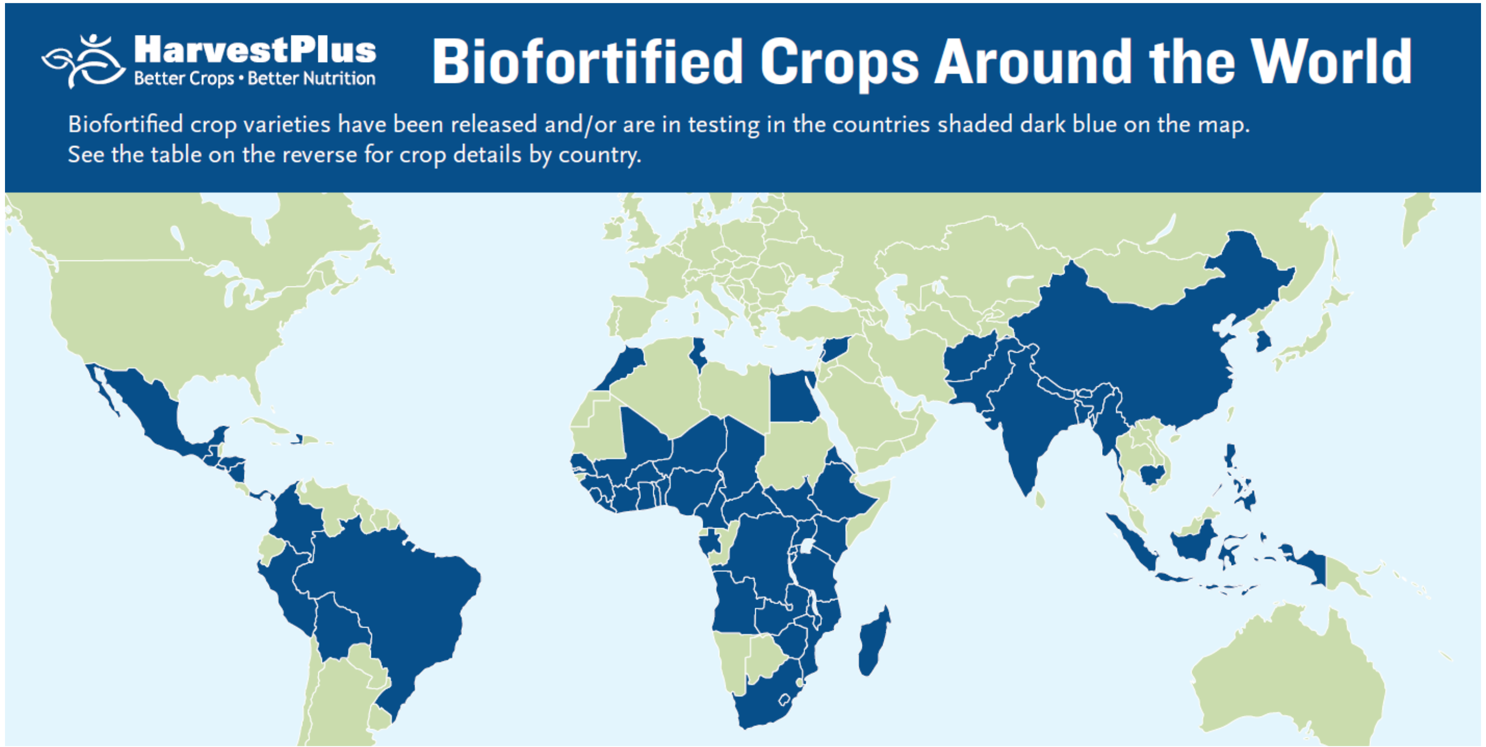
Agronomic:
Foliar spray or soil fertilization

Breeding:
conventional/molecular

Metabolic engineering

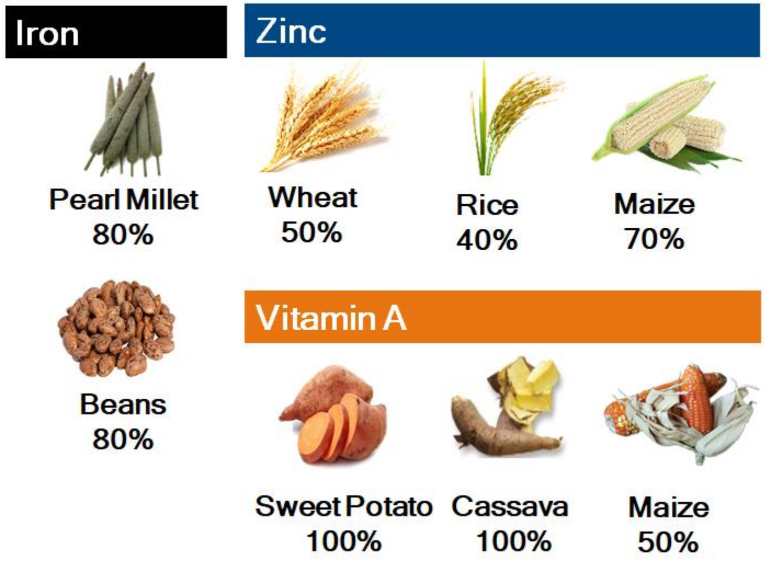
SUSTAINABLE SOLUTIONS!

Reaching 48 million smallholder farmers' households



240 varieties in >30 countries

Providing daily nutrition requirement up to:



The BIG FIVE:

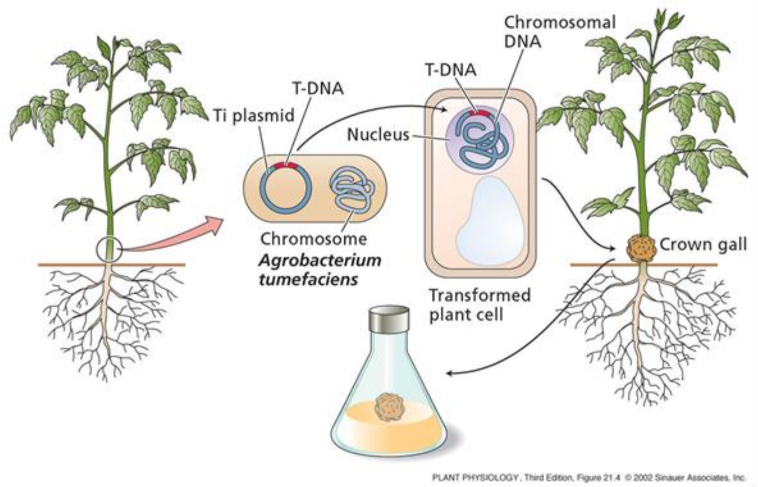
Fe, Zn, vitamin A, *iodine, folates*

Harvest Plus
Biofortification Priority Index

The potential of transgenic approaches

Biofortification through metabolic engineering:
enhancing micronutrient contents by genetic engineering (GE) of vitamin/mineral pathways

How is GE performed, what are the consequences?



Natural process
Widespread!

Pros and Cons: GE versus breeding

Issue		GE	Breeding
Tissue-specificity	Comparative Advantages of GE	**Ability to control tissue-specificity**	No adequate control on tissue-specificity
Source of genetic material		Introduction of genes across species barrier possible	Restricted to sexually compatible gene pool
Time consumed		**Results obtained in limited number of generations**	Require many generations
Transfer of untargeted genes		**Transfer of well-defined genes**	Potential transfer of multiple ([illegible]geted) [illegible]
Knowledge on metabolic pathways	Comparative Advantages of Breeding	Requires sufficient knowled[illegible] metabolic pathway[illegible]	[illegible]owledge of metabolic pathways not required
Enhanced knowledge on micronutrient metabolism		Limited potential to discover new genes involved	Ability to reveal new genes involved

Combine their power!

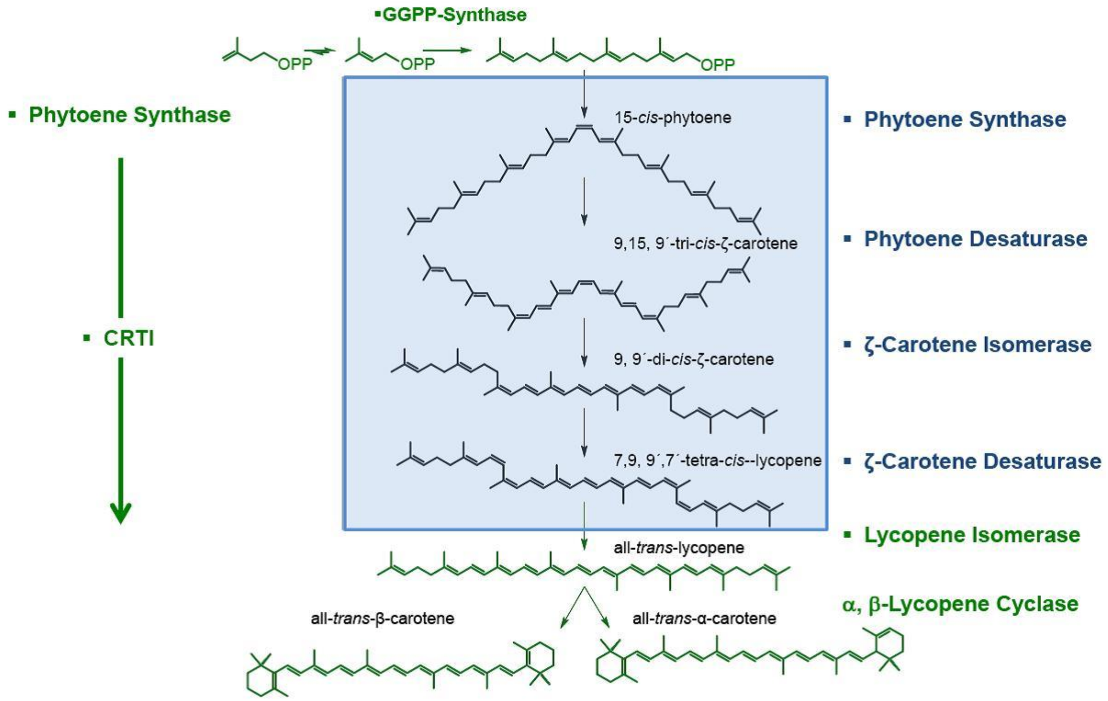
Bouis *et al.*, CAST, Issue Paper 69, Chapt.6, Oct 2020

Golden Rice, 21 years since publication

Engineering the Provitamin A (β-Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm

Xudong Ye,[1]*† Salim Al-Babili,[2]* Andreas Klöti,[1]‡ Jing Zhang,[1] Paola Lucca,[1] Peter Beyer,[2]§ Ingo Potrykus[1]§

www.sciencemag.org SCIENCE VOL 287 14 JANUARY 2000



Nobel laureates' & Academies' support, FDA approved
Still not commercialized (planned The Philippines/Bangladesh)

Advantages of metabolic engineering vs. conventional breeding

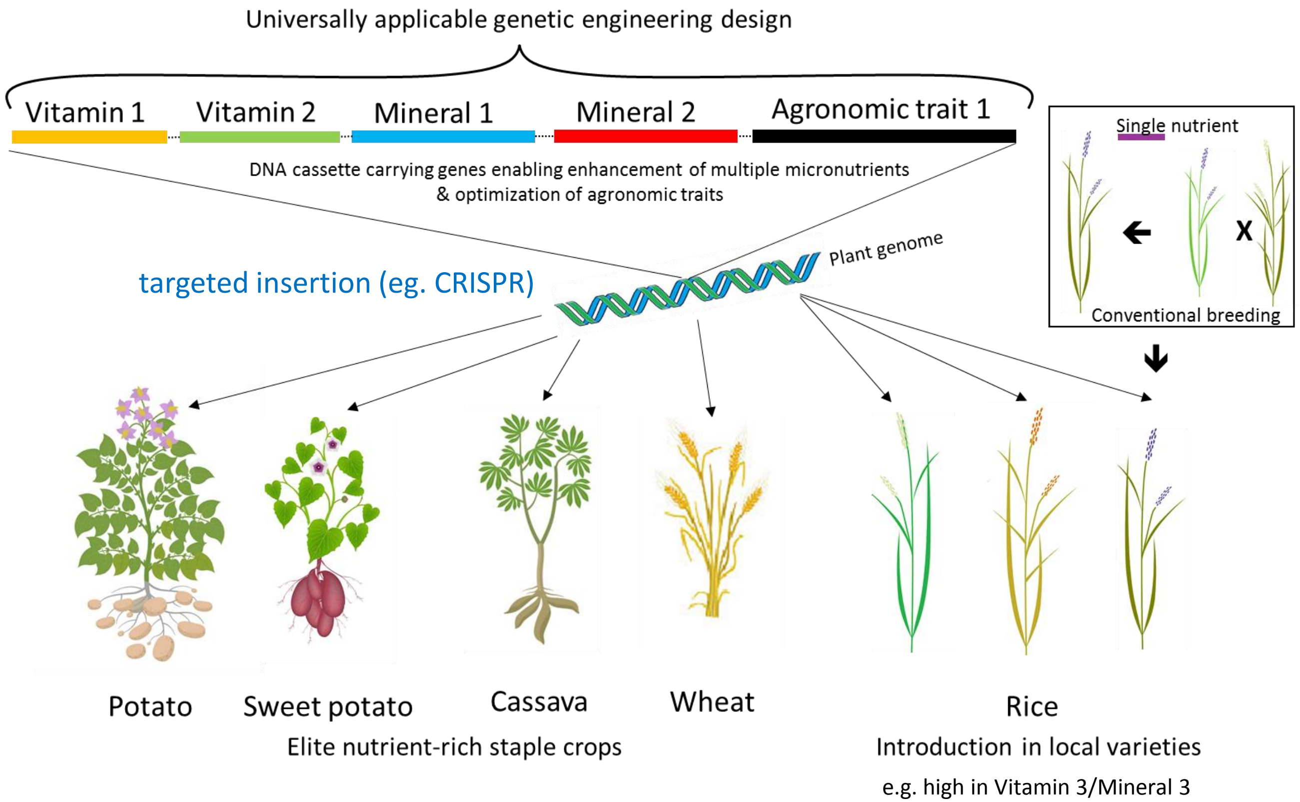
- Address *single nutrients* that are *impossible with conventional* breeding (e.g. provitamin A in rice)
- Achieve *higher densities for single nutrients* than are possible to achieve with conventional breeding (e.g. folates in rice/potato)
- *Easier and faster combination of multiple nutrients* in one crop (e.g. Fe/Zn + vitB9 in rice)
- *Combination of nutrient traits with superior agronomic traits:*

  balance quantity (yield/resilience) & quality traits

Van Der Straeten *et al.*, *Nature Comm.*, 2020

Multi-biofortification through metabolic engineering:

simultaneous enhancement of vitamin/mineral pathways *plus introduction of agronomic traits*



Van Der Straeten *et al.*, *Nature Comm.*, 2020

- Enhanced content of multiple micronutrients
- Enhanced agronomic performance
- Significantly lower fertilizer/pesticide use, improve WUE

- Time saving in terms of realization!
- *Raise acceptance: public funding is key, education on GE essential*

Rational combination of technologies
+ nutrition education and dietary diversification!

Case study folate deficiency:

1 of 5 key micronutrient problems on the globe

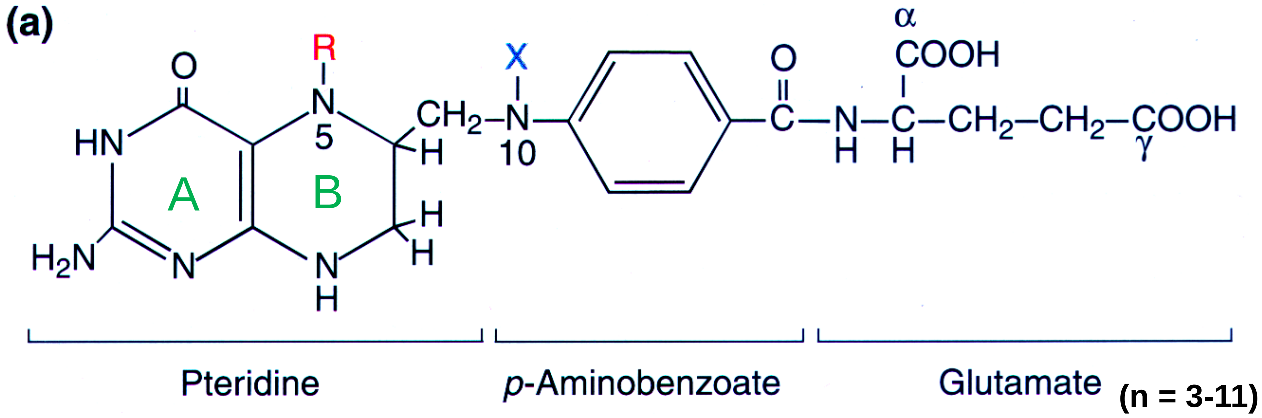
Centers for Disease Control and Prevention (CDC):

- 5 key micronutrient problems:

deficiencies in Fe, Zn, vitamin A, iodine, and folates

- Fe + folates: anemia, affecting over 2 billion people

Folate chemistry



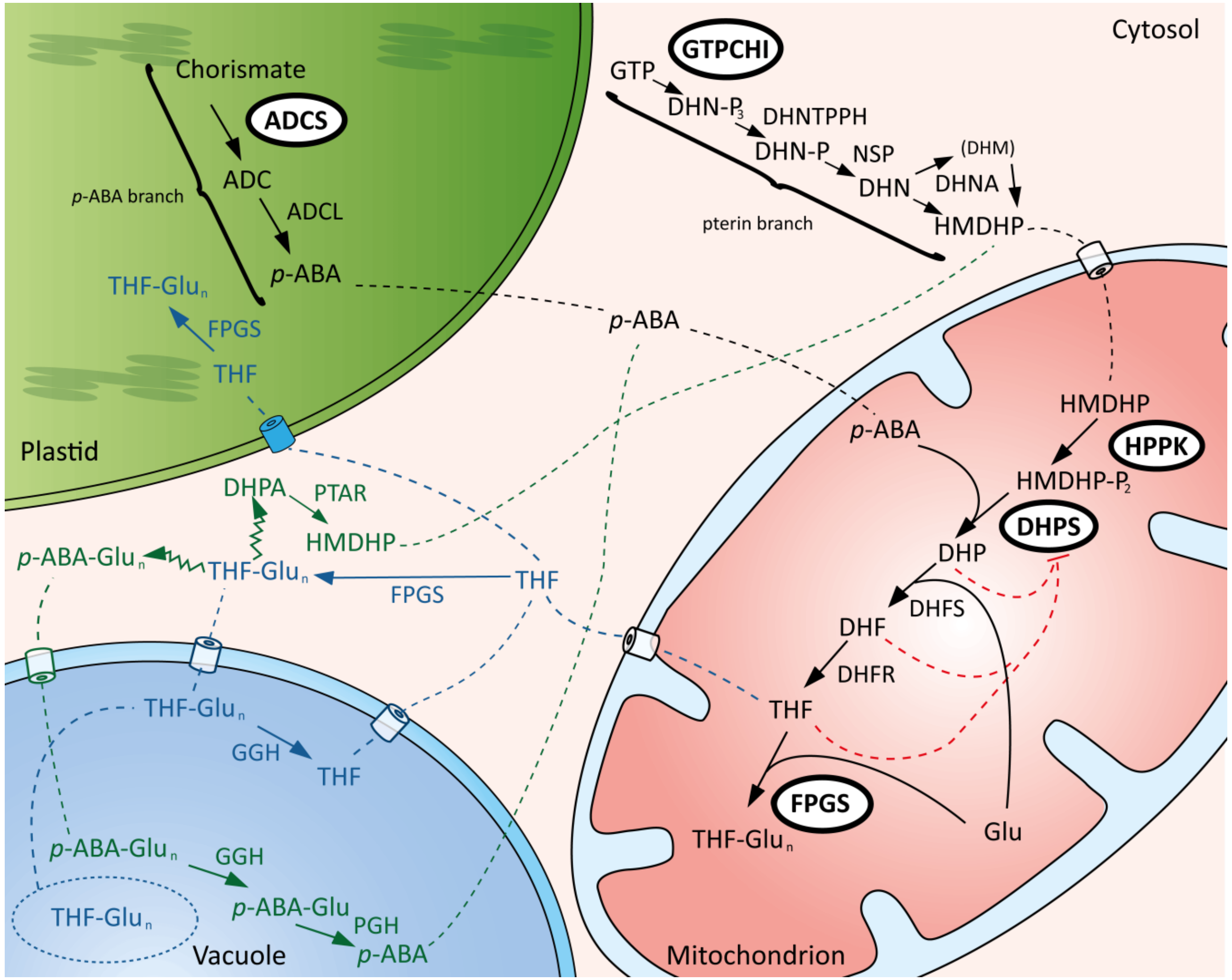
Folate	R	X
THF	H	H
10-Formyl-THF	H	CHO
5-Formyl-THF	CHO	H
5-Methyl-THF	CH_3	H
5,10-Methenyl-THF	=CH−	
5,10-Methylene-THF	$-CH_2-$	

stabilizing → (5-Formyl-THF, 5-Methyl-THF)

Strobbe & Van Der Straeten, *Curr.Opin.Biotech,* 2017

Folate biosynthesis in plants

5 = ADCS
6 = ADCL



1 = GTPCHI
4 = DHNA

7 = HPPK/
8 = DHPS

9 = DHFS
10 = DHFR
/TS
11 = FPGS

Strobbe and Van Der Straeten, *CurrOpinBiotech*, 2017
Gorelova et al., *Sci.Rep.*, 2019

Biochemical functions of folates

Folates function as donors and acceptors of methyl groups in C1 metabolism, *also in humans*:

- Methylation cycle > methylations, Met synthesis
- DNA biosynthesis (G,A,T)

NADPH production, stress tolerance? Fan et al., *Nature*, 2014; Gorelova et al., *TPC*, 2017

Plant specific functions

Consequences of folate deficiency in humans

- Birth defects: NTDs: anencephaly; spina bifida
- Macrocytic anemia, aggravating Fe-deficiency anemia
- *Increased risk for cardio-vascular disease, Alzheimer's, dementia, major depressive disorder*
- *Higher risk for cancer (cervix, lung, colon)*

anencephaly (1/3)

spina bifida (2/3)

Sufficient folate pre-conception can reduce neural tube defects by up to 80%

Strategies for folate biofortification

Target crops: rice and potato

- Marker/metabolomics-assisted breeding: limited to what is offered by nature

  low natural variation in rice (up to 7-fold in 78 cv.)

  low natural variation in potato (4-5-fold in 33 primitive/modern var.)

> *What is theoretically needed?*

Target fold-increase: typical example of the need of GE

Boiled white rice 10µg/100g FW

Boiled potato 20µg/100g FW

50% bioavailable

5µg/100g FW in rice

10µg/100g in potato

Number 2 staple crop

Number 4 staple crop

To reach 600µg in a single serving:

of 150g of boiled white rice: ~80-fold

of 300g of boiled potato: ~20-fold

Folate biofortification

Target crops: rice and potato

- Marker/metabolomics-assisted breeding: limited to what is offered by nature

  low natural variation in rice (up to 7-fold in 78 cv.)

  low natural variation in potato (4-5-fold in 33 primitive/modern var.)

- Metabolic engineering: OE of key limiting enzymes:

  identification of regulatory steps needed

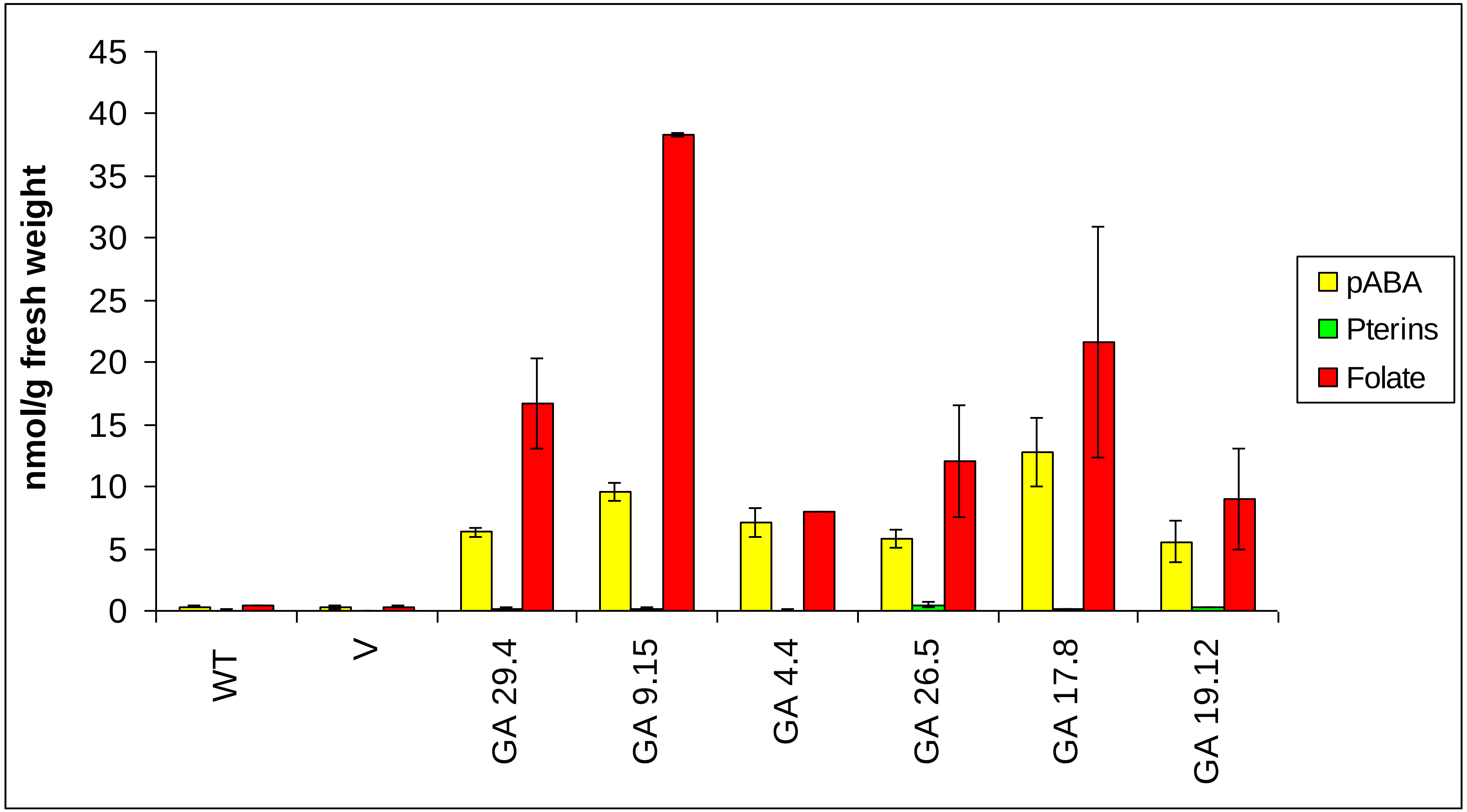
  > simultaneous OE of primary steps in both branches?

  > coupling enzyme?

  > lengthening tail?

100-fold enhancement of folates in GA lines

2 biosynthesis genes
'Push strategy'



Storozhenko *et al.*, *Nature Biotech*, 2007

Endosperm-spec promoters, single T-DNA insertion

Can GA rice meet the RDI?

1700 µg/100g FW

↓ 50% cooking loss

850 µg/100g FW

↓ 50% bioavailability

425 µg/100g FW

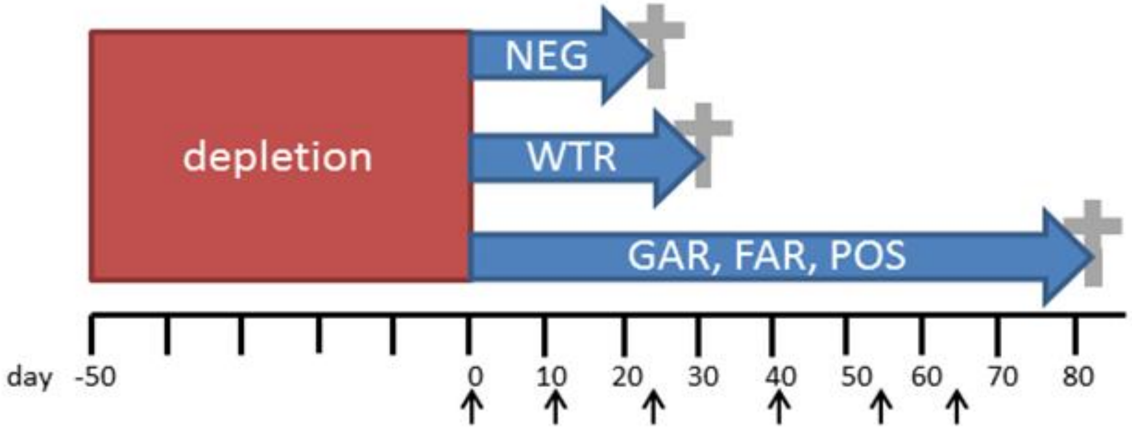
Reaching RDI for <u>pregnant women</u> in 150g of boiled white rice

Storozhenko *et al.*, *Nature Biotech*, 2007

Folate biofortified rice is not only a valuable source of dietary folates…

Folates from metabolically engineered rice: A long-term study in rats

Kiekens *et al*., *Mol. Nutr. Food Res.* 2015



blood sampling from lateral tail vein

Blood markers affected by folate deficiency normalized in individuals receiving ***FA-fortified*** *as well as GA-****biofortified*** *rice (~****3 months in vivo study****)*

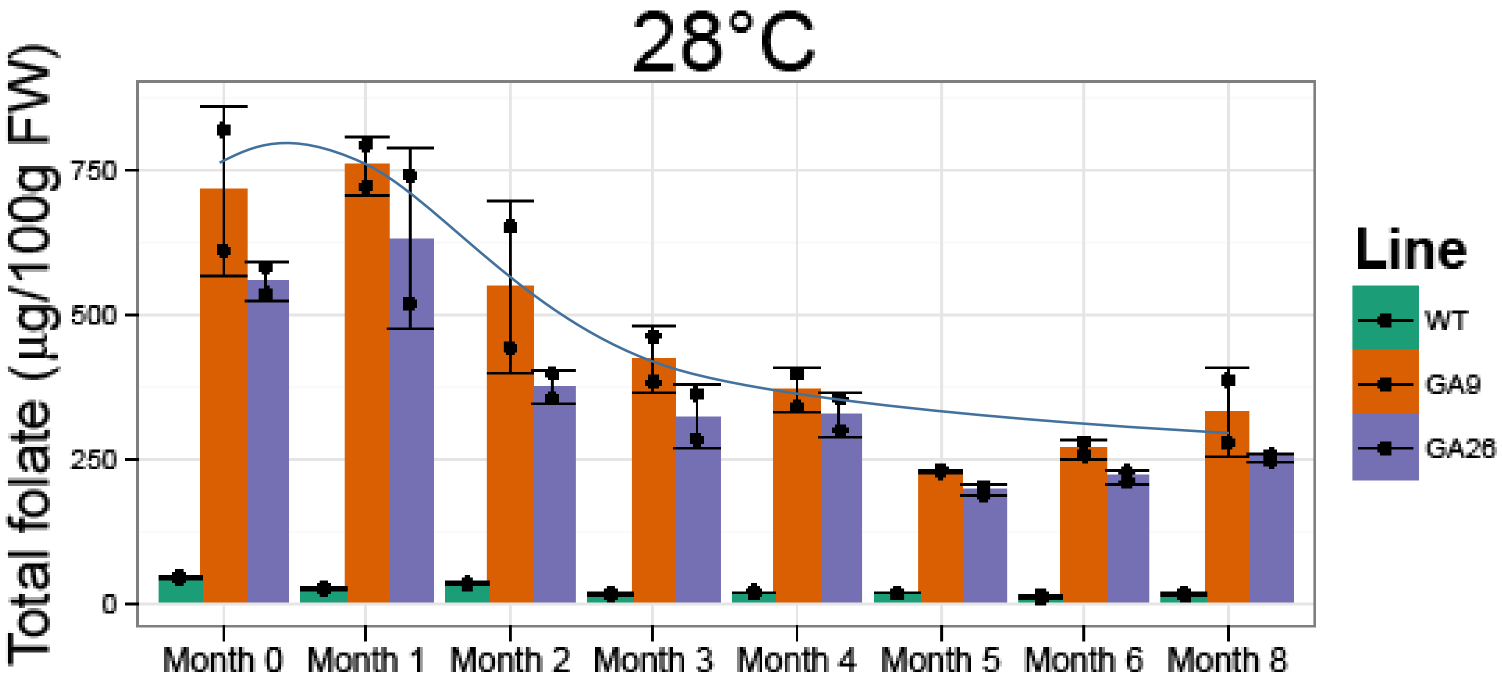
…but also a valuable strategy to reduce folate deficiency & NTDs in China

Health impact in China of folate biofortified rice

De Steur *et al*., *Nature Biotech,* 2010

Impact study using
Disability Adjusted Life Years (DALYs) approach:
Current burden 314000 DALYs , up to 257000 saved annually

Vitamins are labile compounds…



Stored at high T; 50% loss after 4 mo.

Blancquaert *et al.*, *Nature Biotech,* 2015

Postharvest practice!

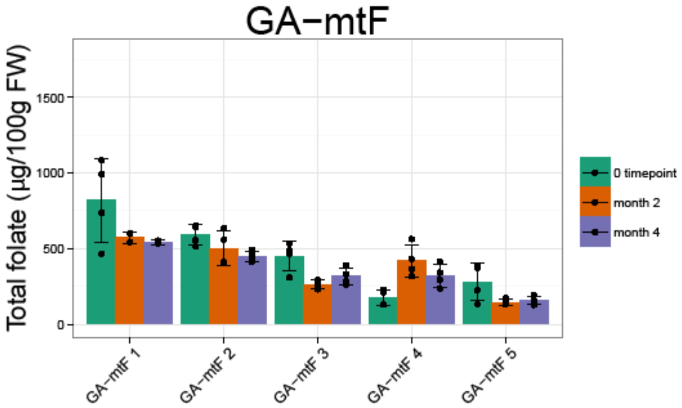
Strategies to enhance folate stability

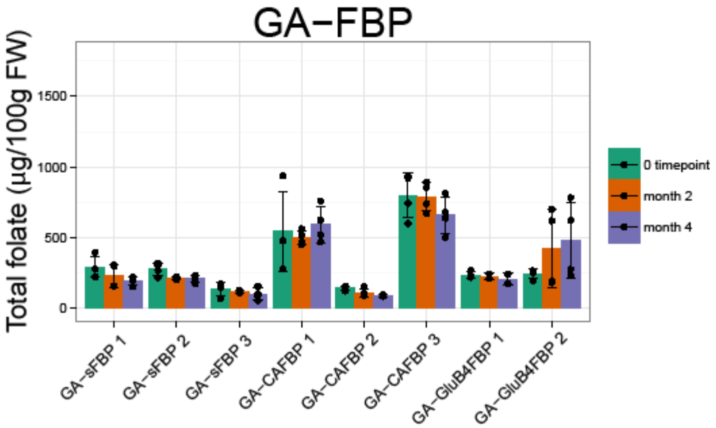
- Increase polyglutamylation (FPGS)
  - Enhance cellular retention through anionic nature of polyglutamate tail
  - Promote binding with folate dependent enzymes

- Complexation with folate binding proteins (FBP)
  - Well described in mammals
  - Creating a sink, advantage!

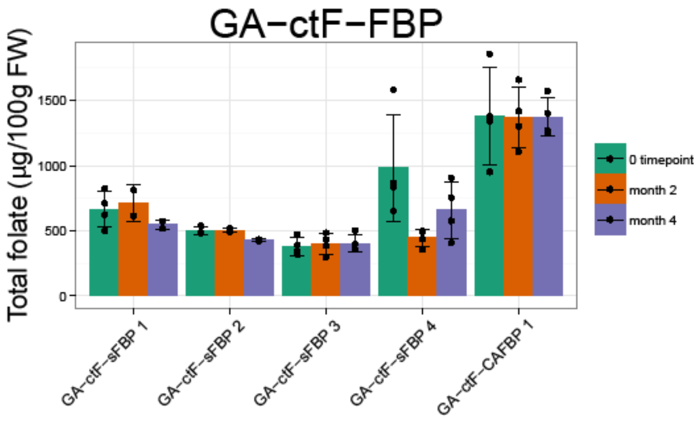
Pull-strategies; Blancquaert et al., *Nature Biotech.,* 2015

Stability GA-FPGS/FBP lines





Stable after 4 mo. at 28°C



Blancquaert et al., *Nature Biotech.*, 2015

Conclusions rice biofortification

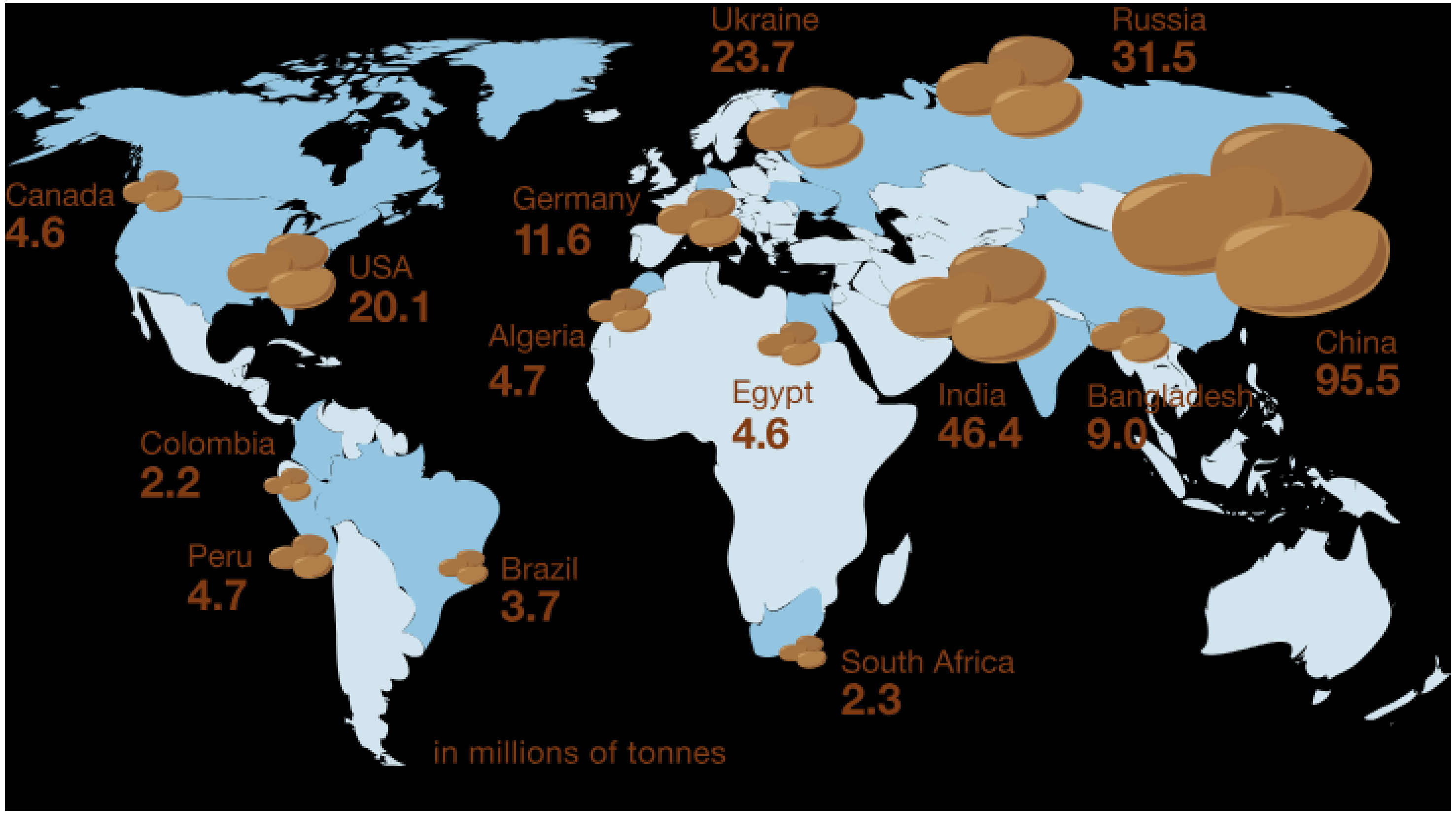
- 2-gene push strategy works, but leaves folates prone to degradation

- Combination of push/pull strategies allows stabilization

- Folates in folate biofortified rice are bioavailable, offering a valuable source of dietary folates

- Biofortified rice offers a sustainable solution to alleviate folate deficiency

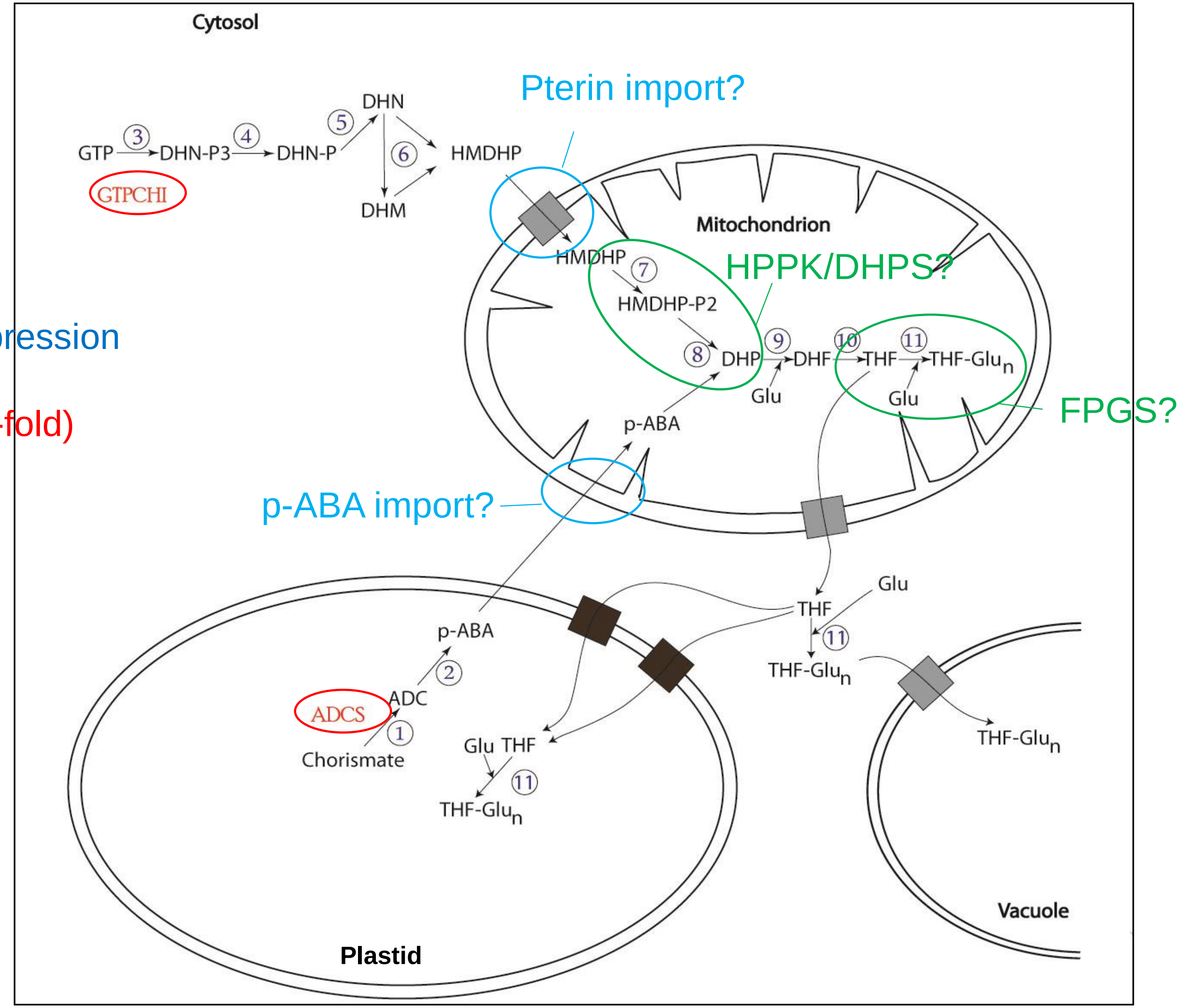
Potato, the number 4 staple crop



Production/consumption rising steadily: China, Algeria, Egypt, Malawi, RSA, Rwanda, Kenya

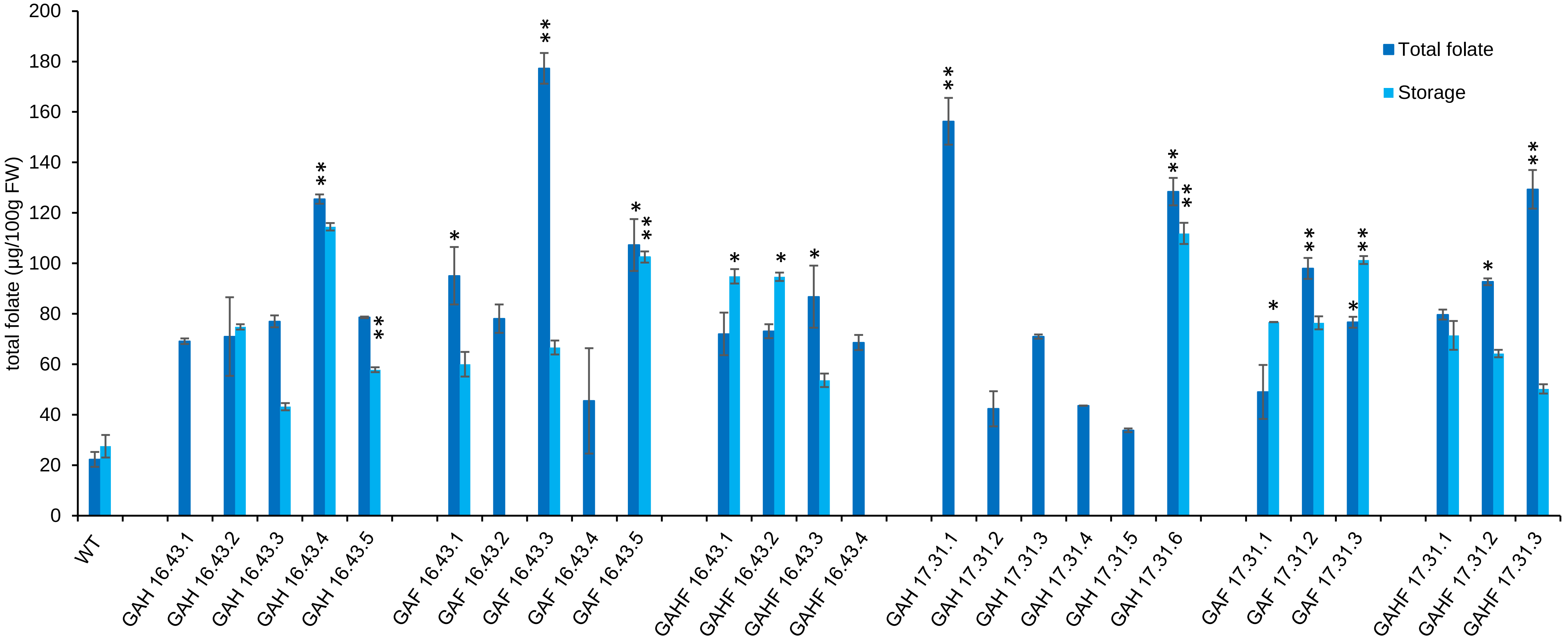
What hampers folate enhancement in potato?

- GTPCHI & ADCS overexpression
- pterin & pABA enhanced
- modest folate increase (2-fold)



Blancquaert *et al*., *J. Exp. Bot.* (2013)

Folate biofortification of potato by tuber-specific expression of 4 folate genes



De Lepeleire & Strobbe *et al.*, *Mol.Plant*, 2018

120d-old; 9 months storage

Proof of concept achieved

385 µg/100g FW

↓ *50% cooking loss*

192 µg/100g FW

↓ *50% bioavailable*

96 µg/100g FW

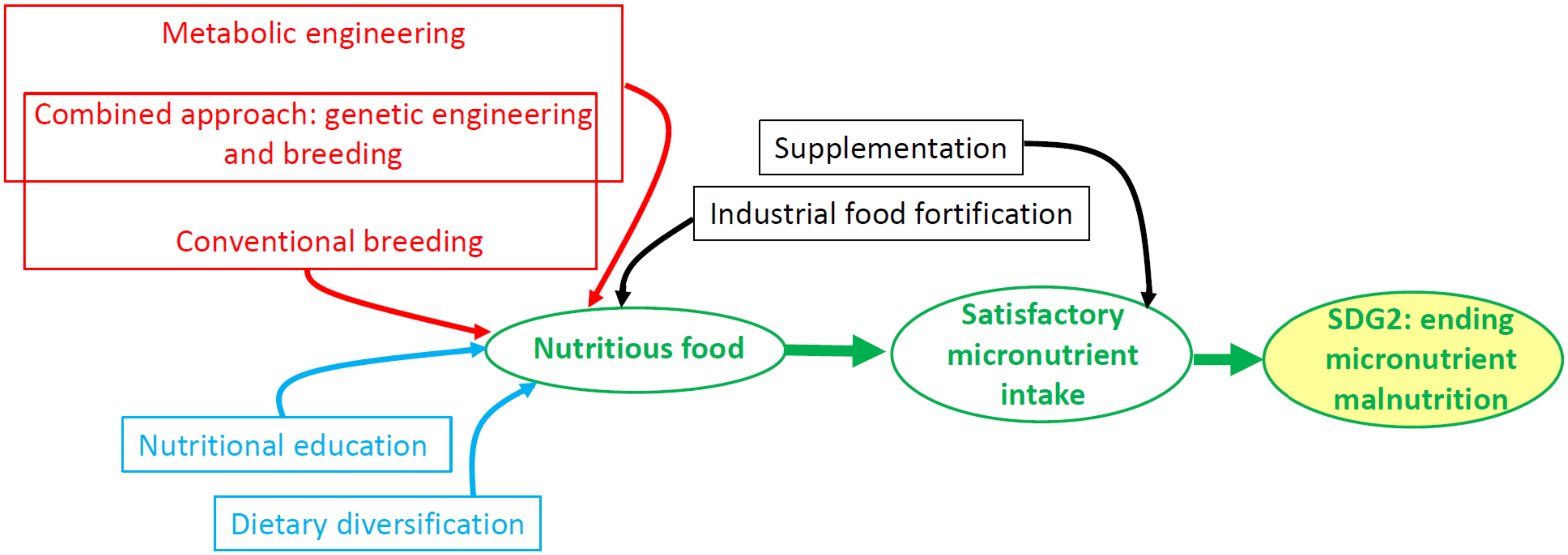
72% of RDI in a single serving
of 300g of boiled potato

48% of RDI 600µg pregnant women in a single serving
of 300g of boiled potato

Regulation is sp. dependent!

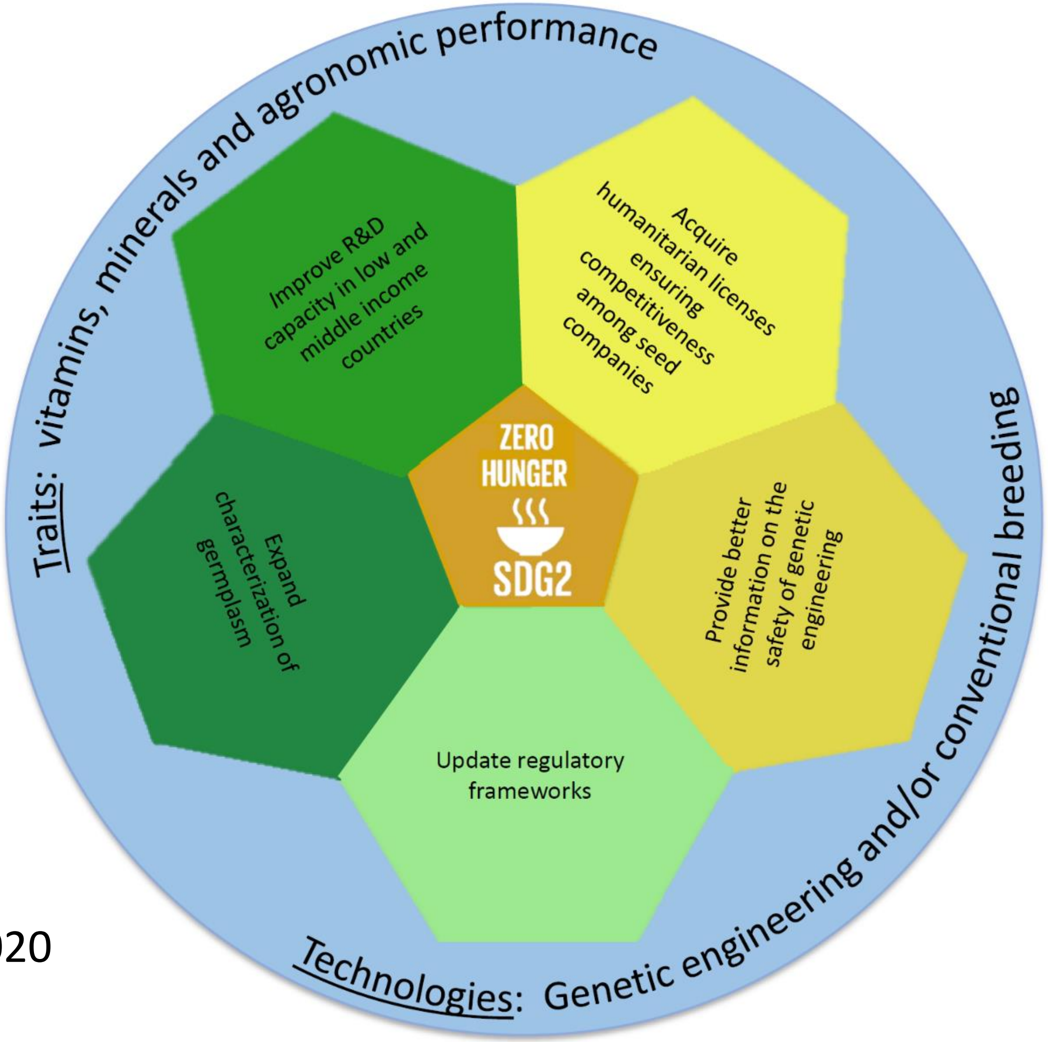
Road map towards ZERO HUNGER

Sustainable approach



Van Der Straeten *et al.*, *NComm*, 2020

Road map towards ZERO HUNGER



Van Der Straeten *et al.*, *NComm*, 2020

Thank you for your attention!

Muito obrigada!

Grazie mille!

Merci beaucoup!

For more information on our work on vitamins and biofortification, see *www.fpb.ugent.be*