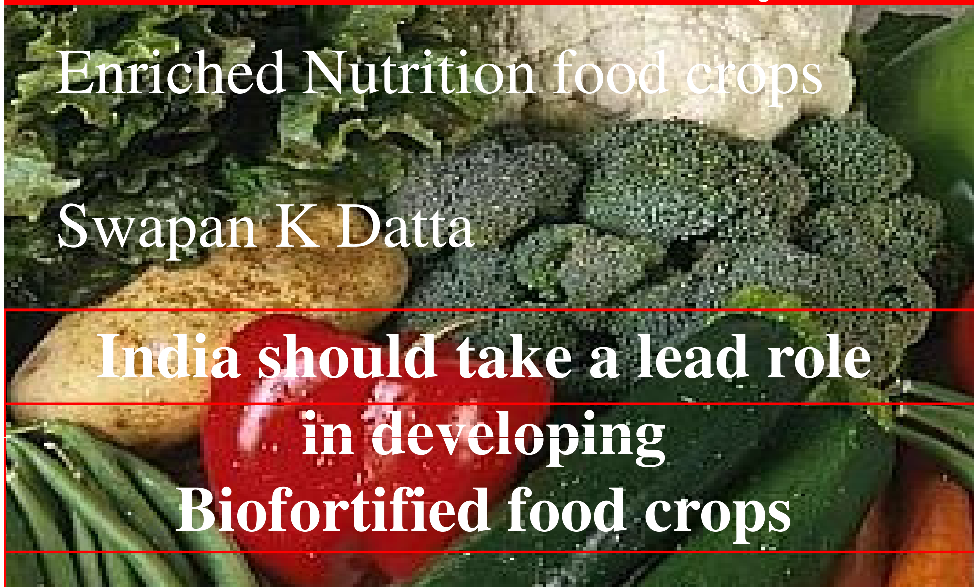
30% Indian population (mostly women and children) are malnourished : Food + Nutrition Security come together & can easily be utilized with PDS



Improved carotenoids & protein-potato (*Ama1*)
Insulin promoting rice
Low P Canola with β-carotene
Vitamin C food crop
High iron rice
β-carotene + Vit E rice
Vitamin E + β-carotene maize

Genetic change resulting from crop domestn. took 10,000 years. Teosinte (L) and corn or maize(R)

Vitamin E enriched corn

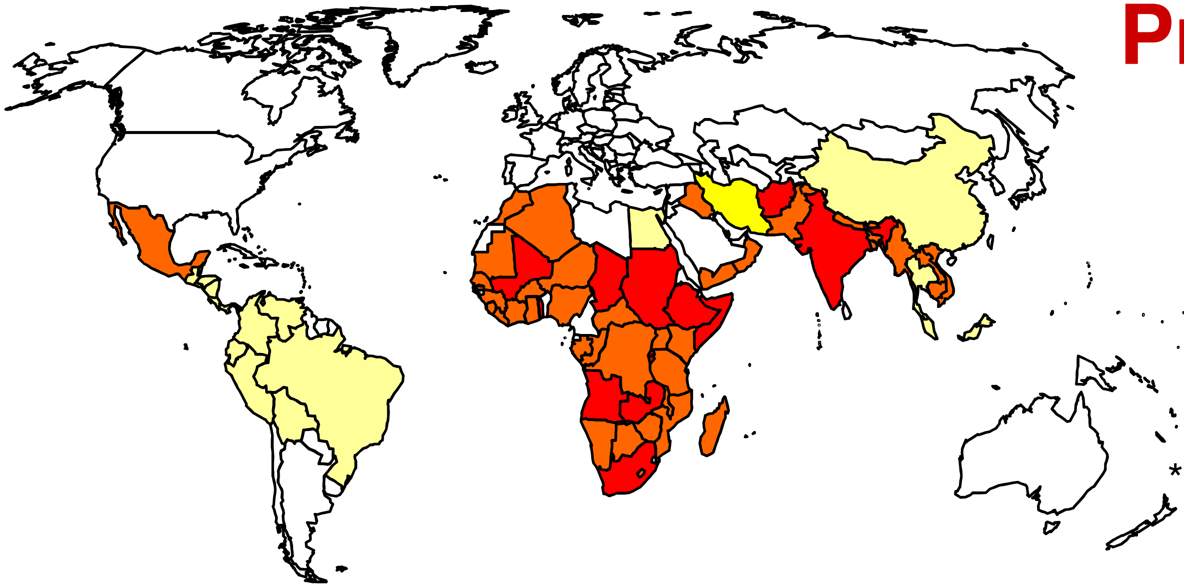
Thomas Edison 1879……

Traditional bulb will be replaced by compact fluorescent light (CFL).
These use up to 75% less power and
last ten times longer, but they cost around $3 each
The most promising alternatives are light-emitting diodes (LEDs).
energy savings of up to 80% and a working life of
45,000 hours. But they are not cheap: around £40 ($56) in Britain.
Gallium nitride-on-silicon LEDs would make commercial sense
A smaller solar powered LED-reading light (cost $15)
LEDs this way really will let in the light.

Nutrition enriched food crop

- Importance of Nutrition enriched crop
- Why genetic engineering to alter the pathways?
- What and how do we understand the pathways
- Can pathways relate to functional gene expression?
- Cross-talk and phenotyping
- Dream Nutrition enriched Crop

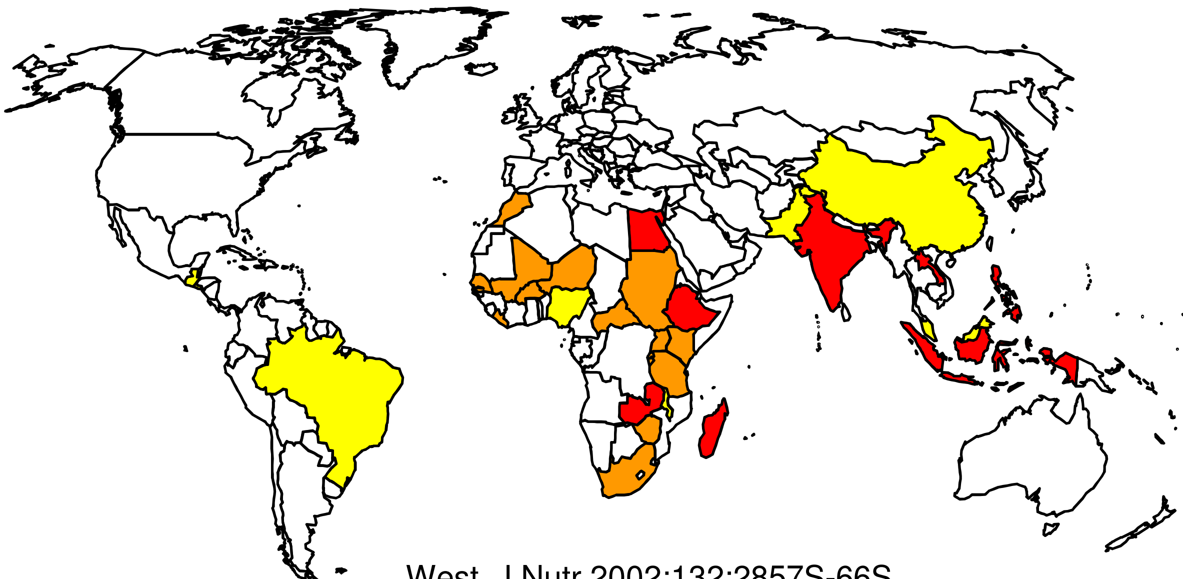
Geographical distribution of vitamin A deficiency



Preschool children

- X ≥1.5 % and VAD ≥15 %
- X ≥1.5 % or VAD ≥15 %
- X =0.5 % to 1.49 % and VAD <15 %
- X <1.5 % and VAD <15 %

* serum retinol concentrations < 0.70 µmol/L or abnormal conjunctival impression cytology, and xerophthalmia (X), all active stages combined



Pregnant women

- VAD ≥20 % and XN ≥1.5 %
- VAD ≥20 % or XN ≥1.5 %
- VAD <20 % or XN <1.5 %

* serum or breast milk retinol concentrations <1.05 µmol/L and maternal night blindness, based on extant data for either or both indicators

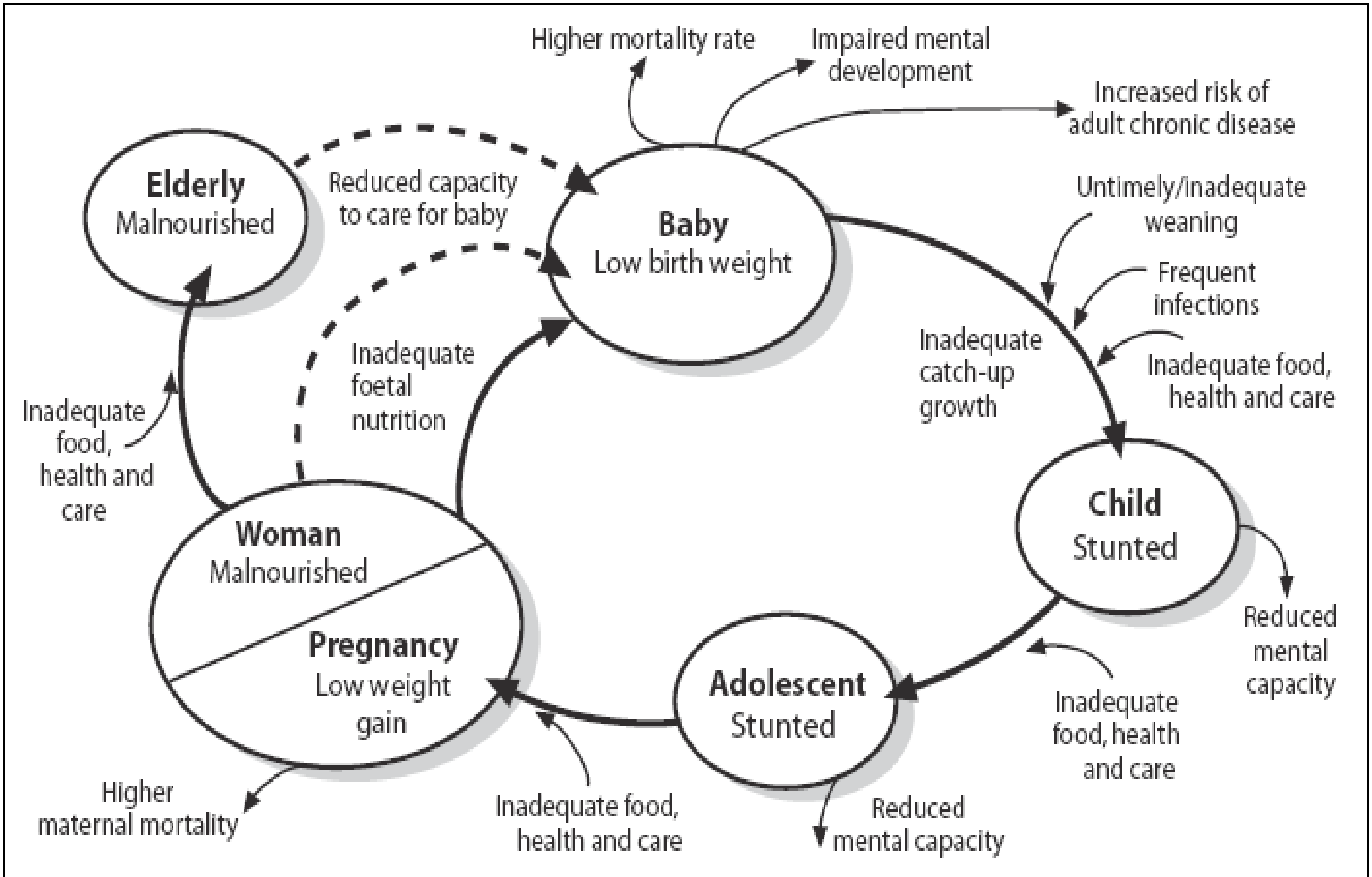
West. J Nutr 2002;132:2857S-66S.

GLOBAL FOOD SECURITY AND MALNUTRITION

More rice and more nutritional rice must be produced on less land with less water and with less harmful chemicals

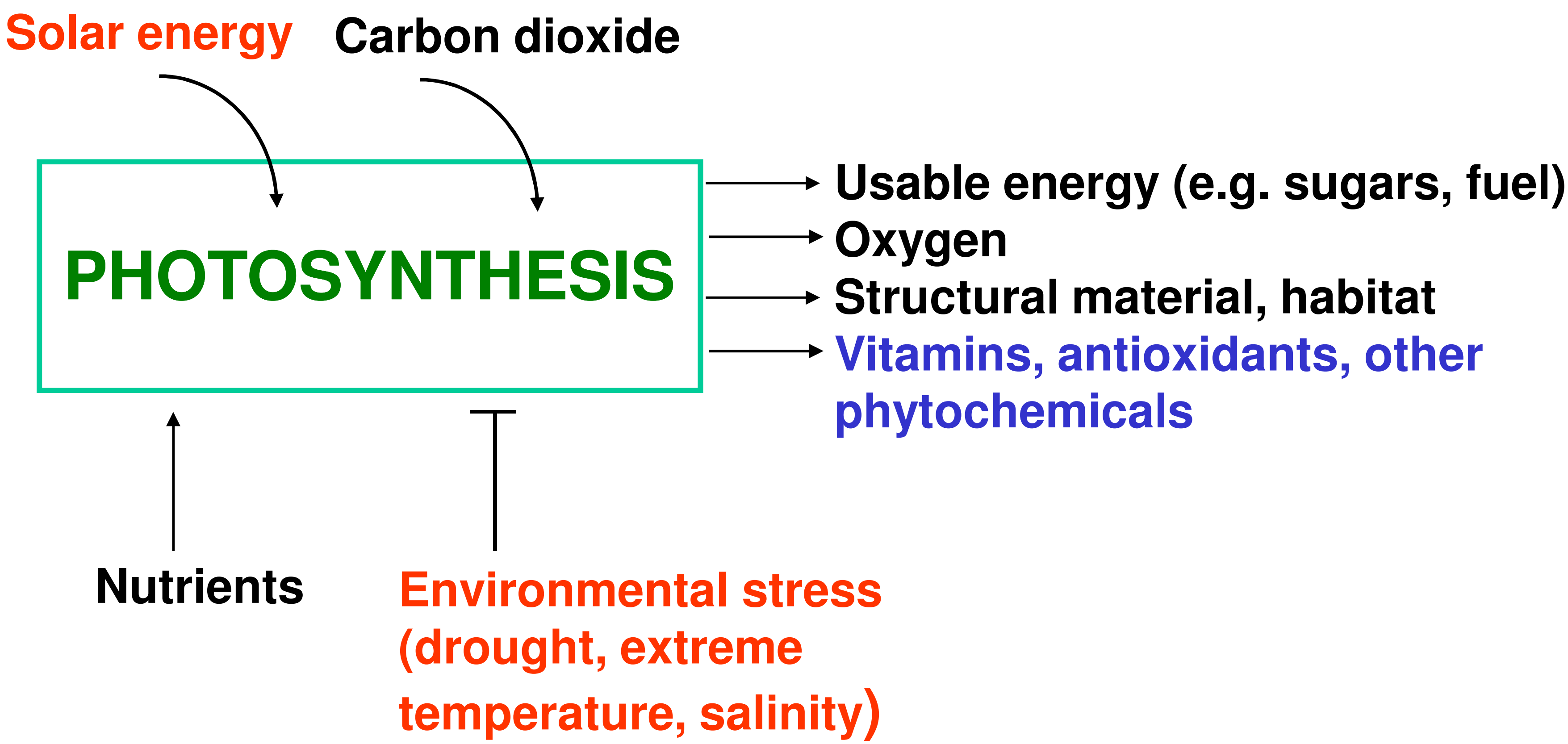
- **1.1 billion are absolutely poor with incomes < 1U$ day**
- **2.0 billion are marginally better off**
- **840 million people are food insecure**
- **200 million malnourished children**
- **400 million have acute iron deficiency**
- **125 million are affected by a lack of vitamin A**
- **Only 4% rice of the world supply is non-traded internationally**
- **Many of 8 billion people on the earth by 2020 will live outside the market driven supply of food**

RANGE OF MALNUTRITION

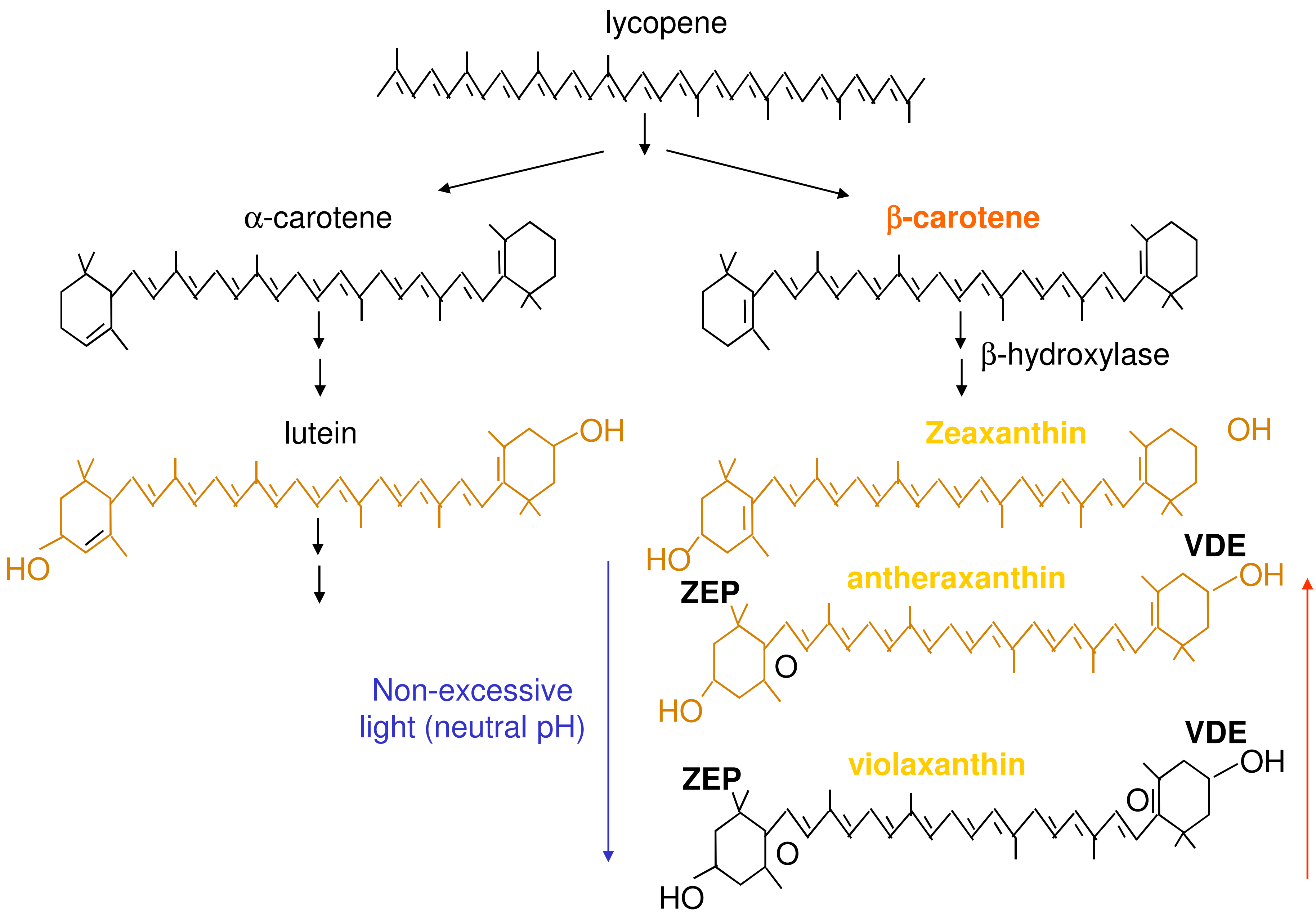


Effects of malnutrition throughout the life cycle.

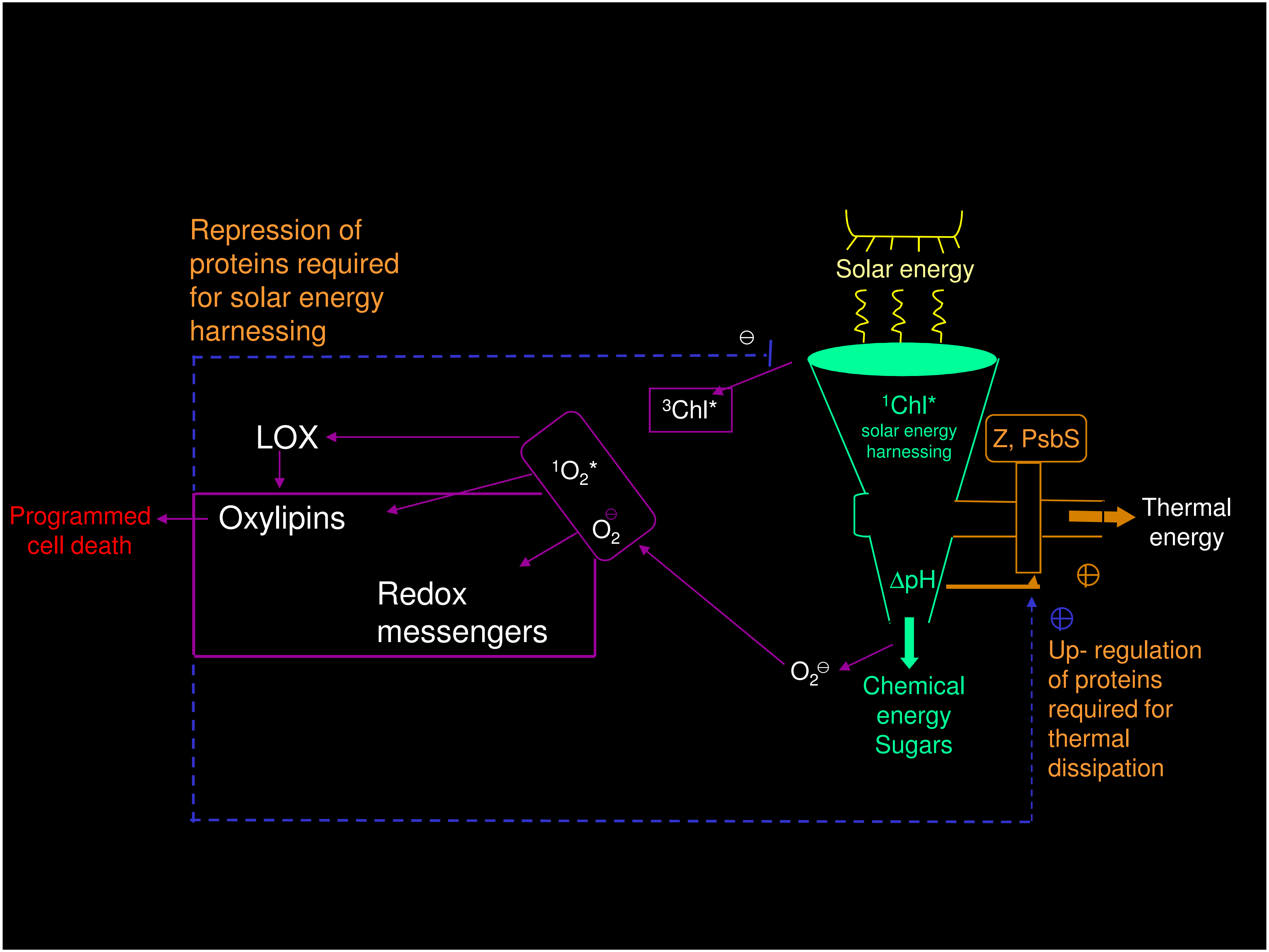
Source: SCN Publications



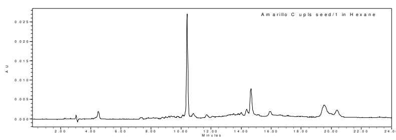
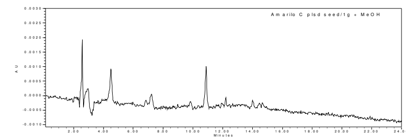
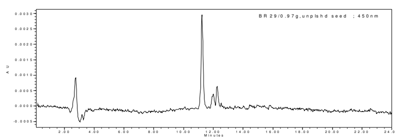
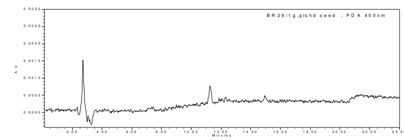
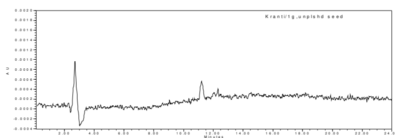
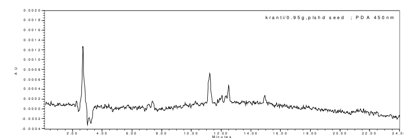
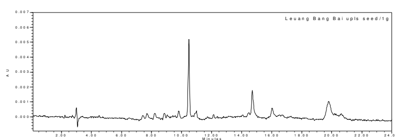
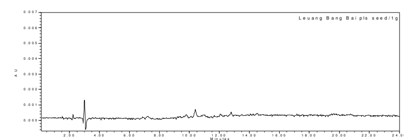
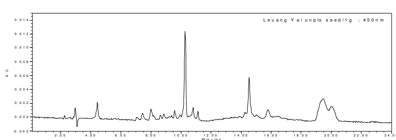
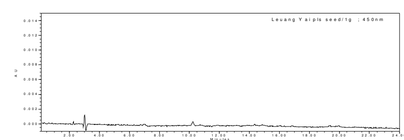
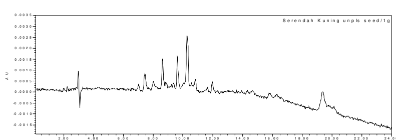
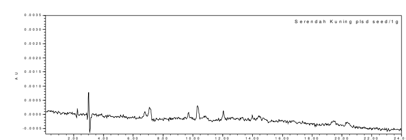
Science, 13 December 2002



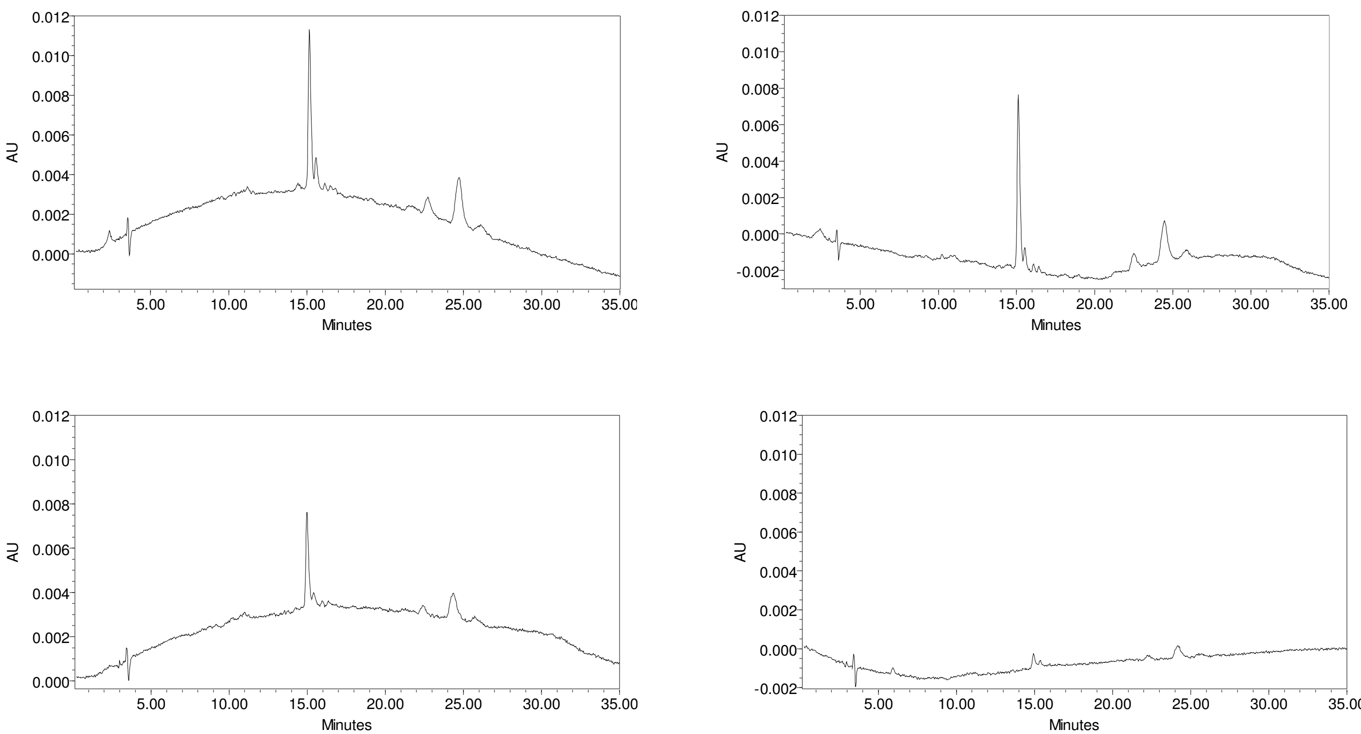
lycopene
α-carotene
β-carotene
β-hydroxylase
lutein
OH
HO
Zeaxanthin
OH
HO
VDE
antheraxanthin
ZEP
OH
O
HO
Non-excessive
light (neutral pH)
VDE
violaxanthin
ZEP
OH
O
O
HO


Repression of proteins required for solar energy harnessing
⊖
Solar energy
^{3}Chl*
LOX
^{1}Chl*
solar energy harnessing
Z, PsbS
$^1O_2$*
Programmed cell death
Oxylipins
$O_2^{\ominus}$
Thermal energy
ΔpH
⊕
Redox messengers
⊕
$O_2^{\ominus}$
Chemical energy Sugars
Up- regulation of proteins required for thermal dissipation

Genotype screening for the carotenoids in brown and milled rice

Variety Name	Country of Origin	HPLC Chromatograph of Unpolished Seed	HPLC Chromatograph of Polished Seed
Amarillo C	Cuba		
BR 29	Bangladesh		
Kranti	India		
Leuang Bang Bai IRGC# 47858	Thailand		
Leuang Yai 74-4-20 IRGC# 44159	Thailand		
Serendah Kuning IRGC# 47740	Indonesia		

Gradual Decrease of Carotenoids with the Increasing of Polishing Time (SECONDS)



Golden Rice (BR29) developed at IRRI is now in Bangladesh soil
Syngenta-Golden Rice (GR2) is now in field at Louisiana, USA

3.0-9.1 µg/g, DH homozygous lines developed

Commercial right of GR remains with Syngenta

Datta K et al PBJ, 2003/2005,2006
Parkhi et al MGG, 2005,2006
Rai et al 2003,2006
Ye et al Science, 2000
Painie et al Nature Biotech, 2005

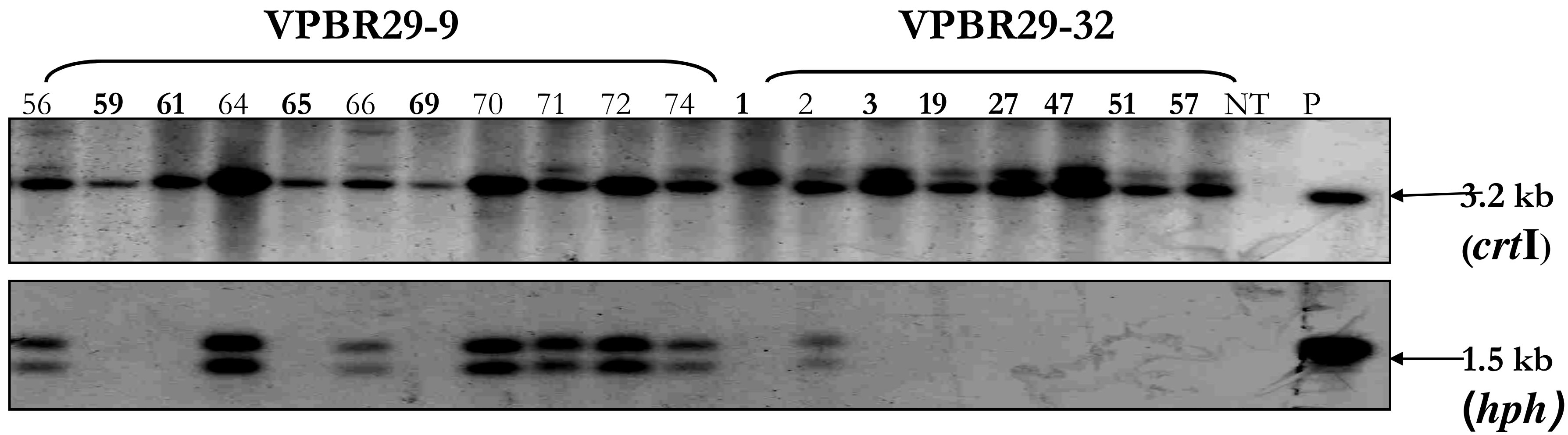


Fig 3

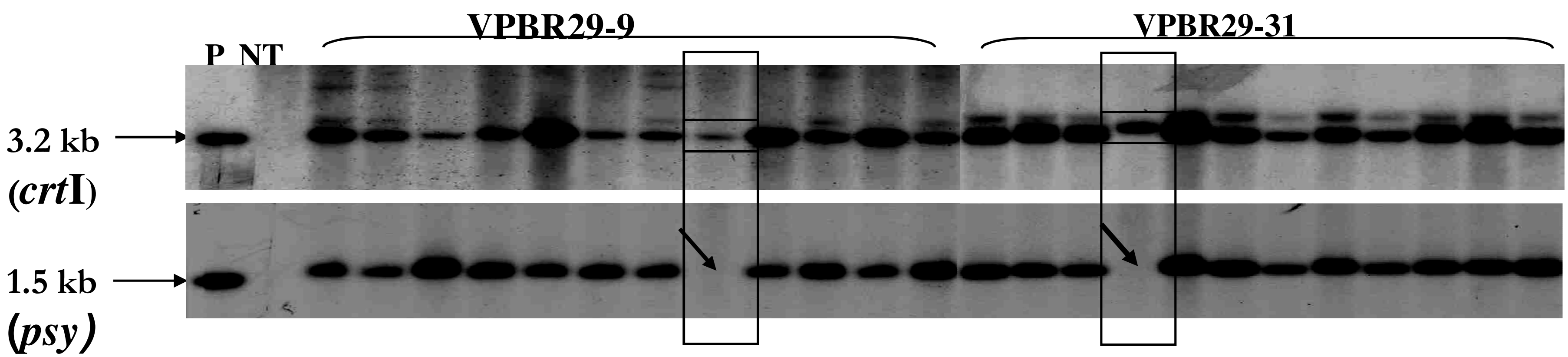
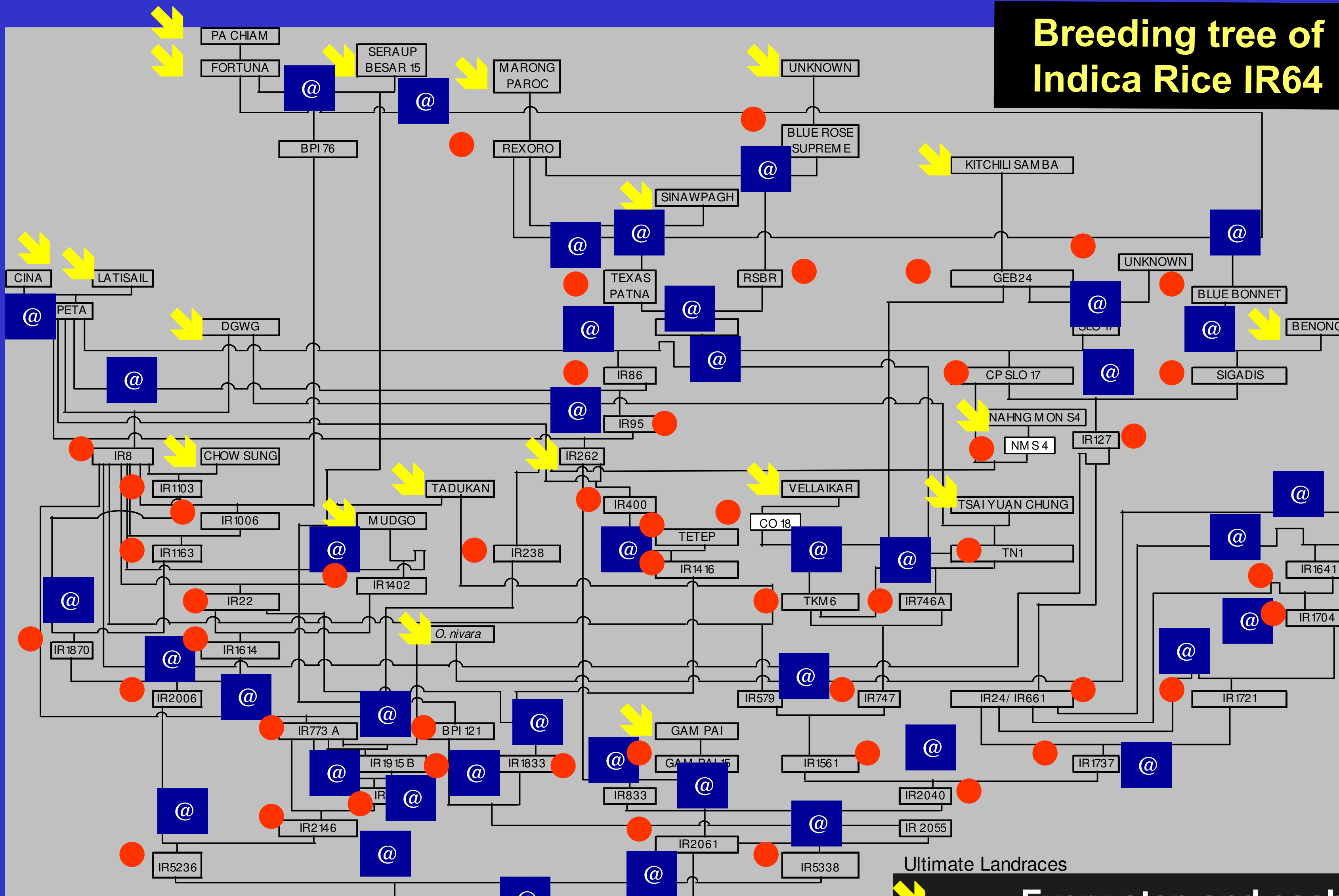


Fig 4

Golden BR29 rice without a marker gene (Mol Gen Genomics 2005)

Breeding tree of Indica Rice IR64

Traditional breeding leads to massiv and uncontrolled modifications of the genome!



Ultimate Landrace

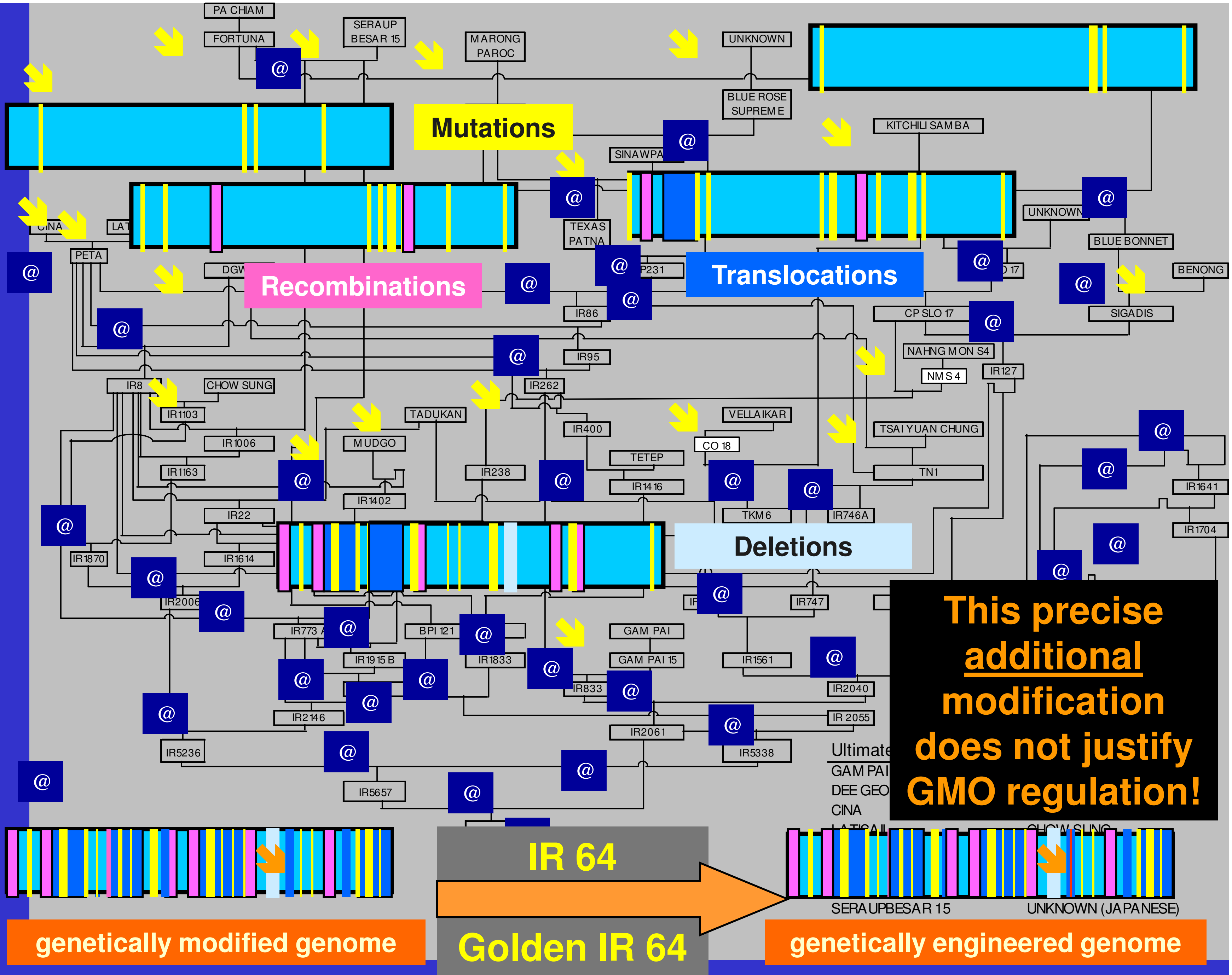
New varieties

Crossing & selection

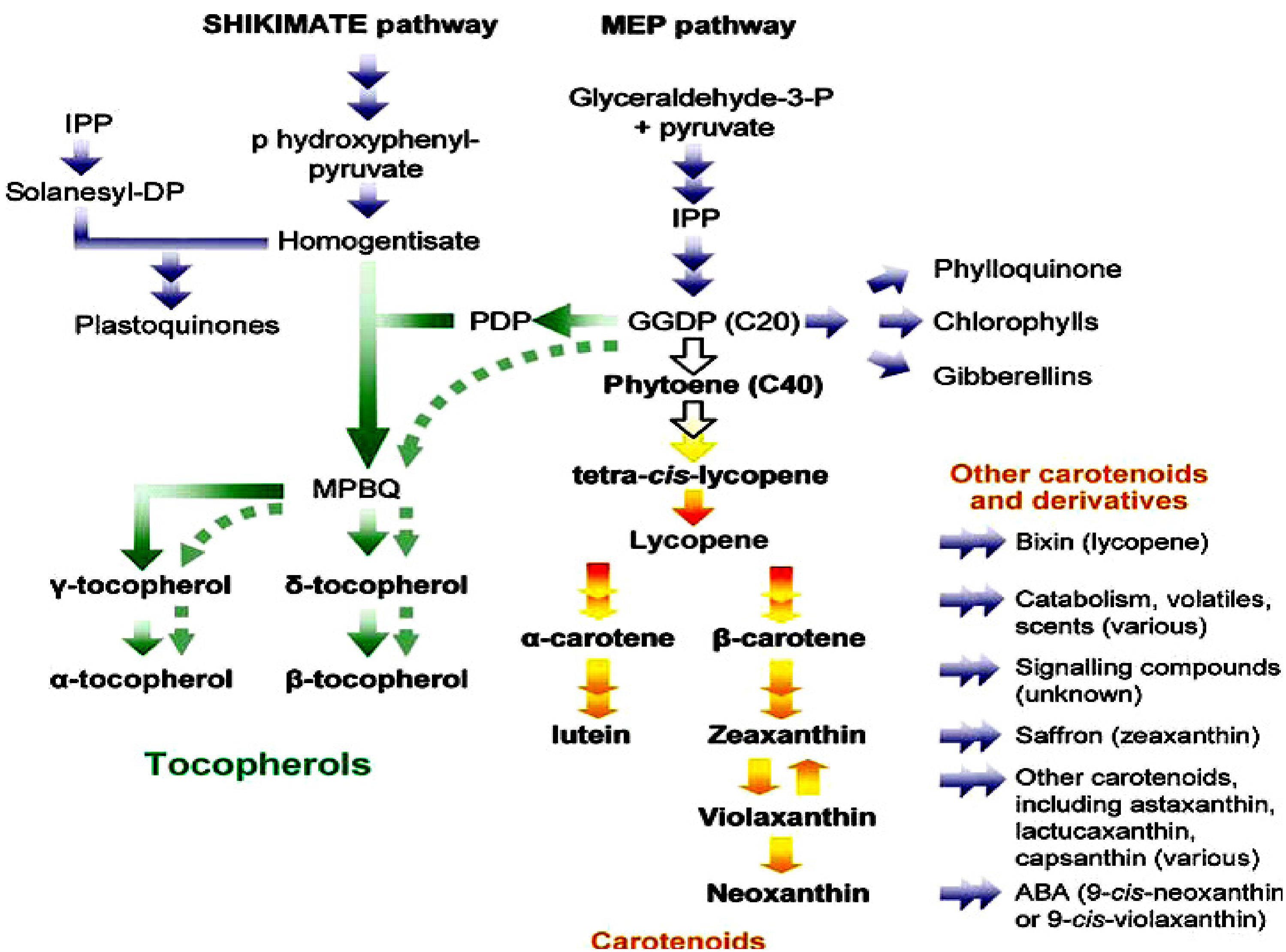
IR 64

Every step and each component is unpredictable and leads to uncontrolled genome alterations!

Golden IR64 differs from IR64 by a relaively tiny, carefully studied, precisely described recombination.

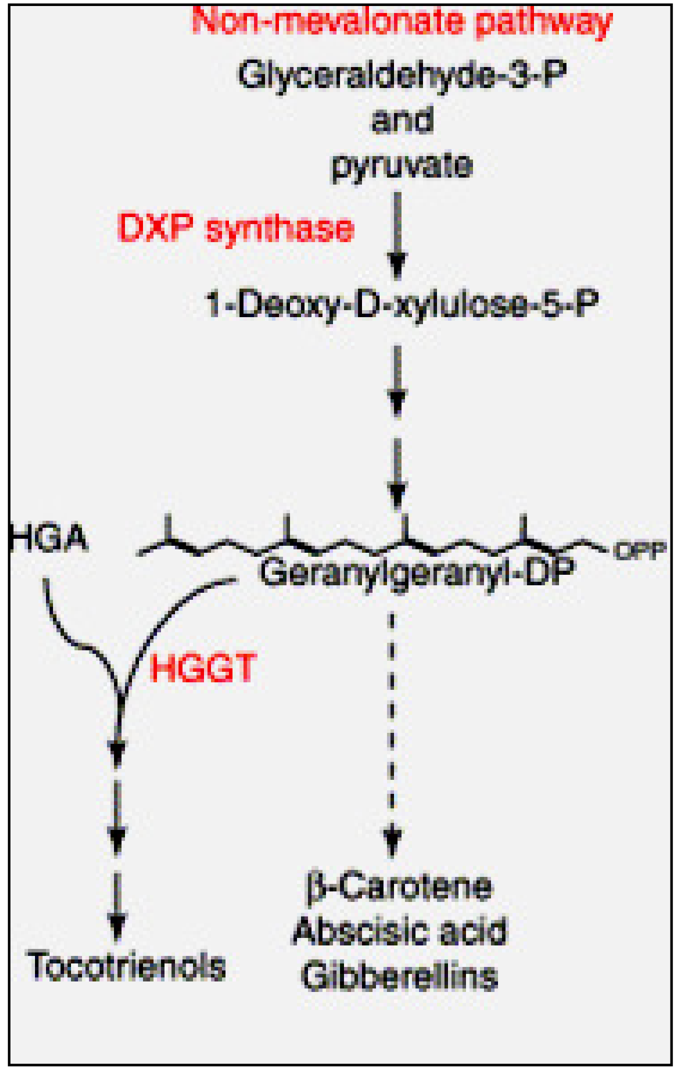


Biosynthetic pathway of Tocopherols & Tocotrienols

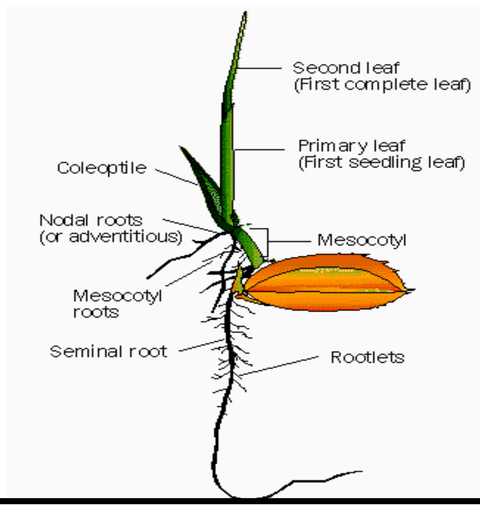


Vitamin E- Maize

- **HGGT** catalyzes an analogous reaction to HPT, only it is highly specific for GGDP whereas HPT uses PDP as its prenyl substitute.
- Results from the expression of **barley HGGT** in transgenic plants suggest that this enzyme has strong substrate **specificity for geranylgeranyl diphosphate, rather than phytyl diphosphate**.
- Expression of HGGT enzyme in tobacco calli and Arabidopsis leaves resulted in accumulation of Vitamin E antioxidants in the form of tocotrienols ,principally as **γ-Tocotrienols**, and generated little or no change in the content of Tocopherols (Cahoon et al, 2003)
- Barley **HGGT** gene was over-expressed in maize seeds, leading to a **20-fold increase** in tocotrienol level, which translated to an eight-fold increase in total tocols (tocopherols and tocotrienols) (Cahoon et al, 2003).



Nutrittion biofortified rice



↓ **PS (*psy*)**

Phytoene (1)

↓ **PDS (*crtI*)**

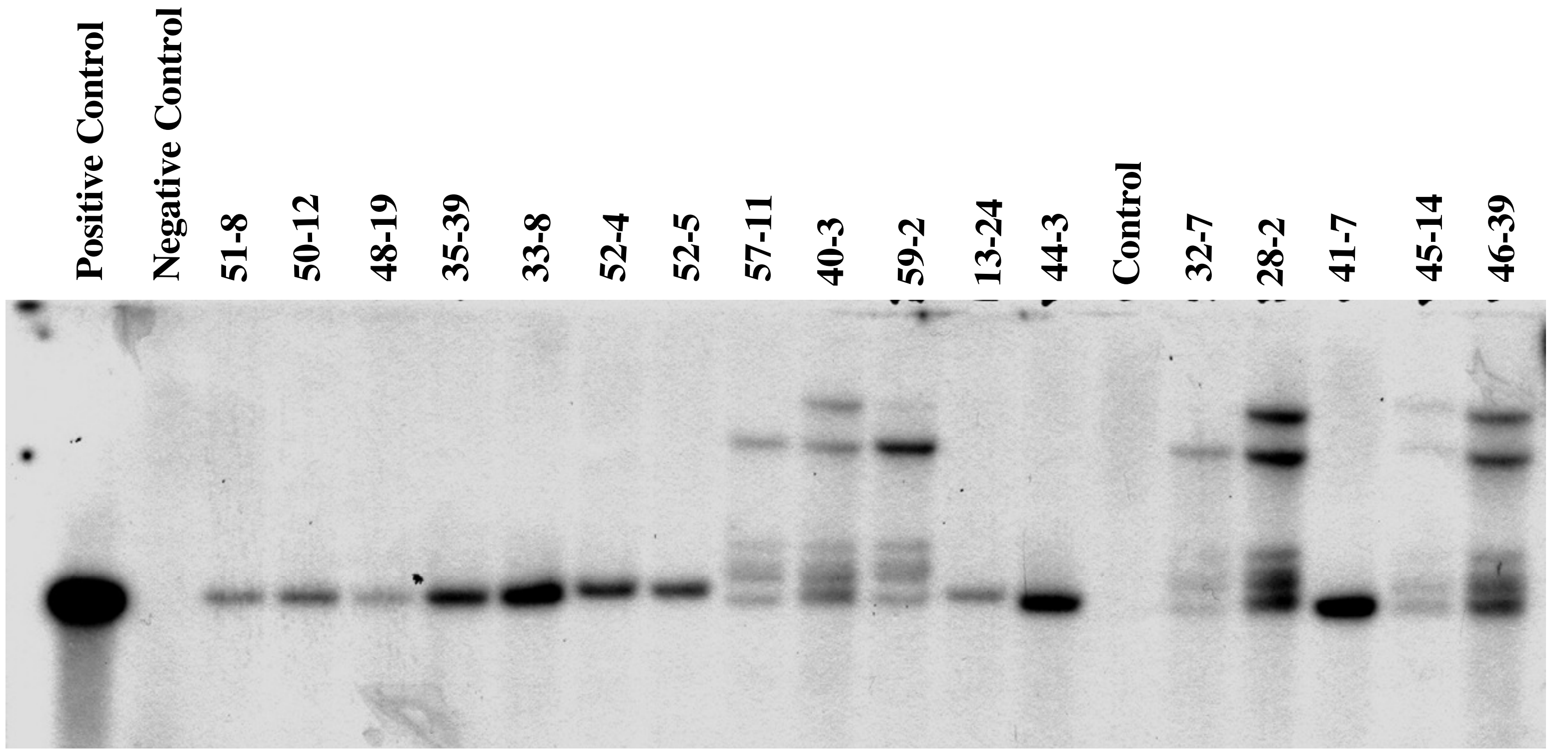
Lycopene (2)

↙ ↘ **LC (*lyc*)**

α-carotene **β-carotene (3)**

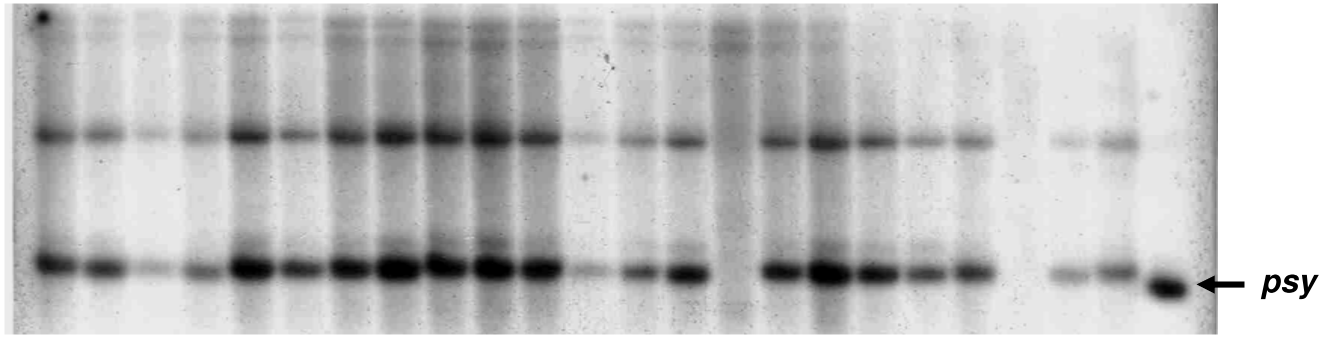

RRI


IR 64
(GOLDEN RICE)

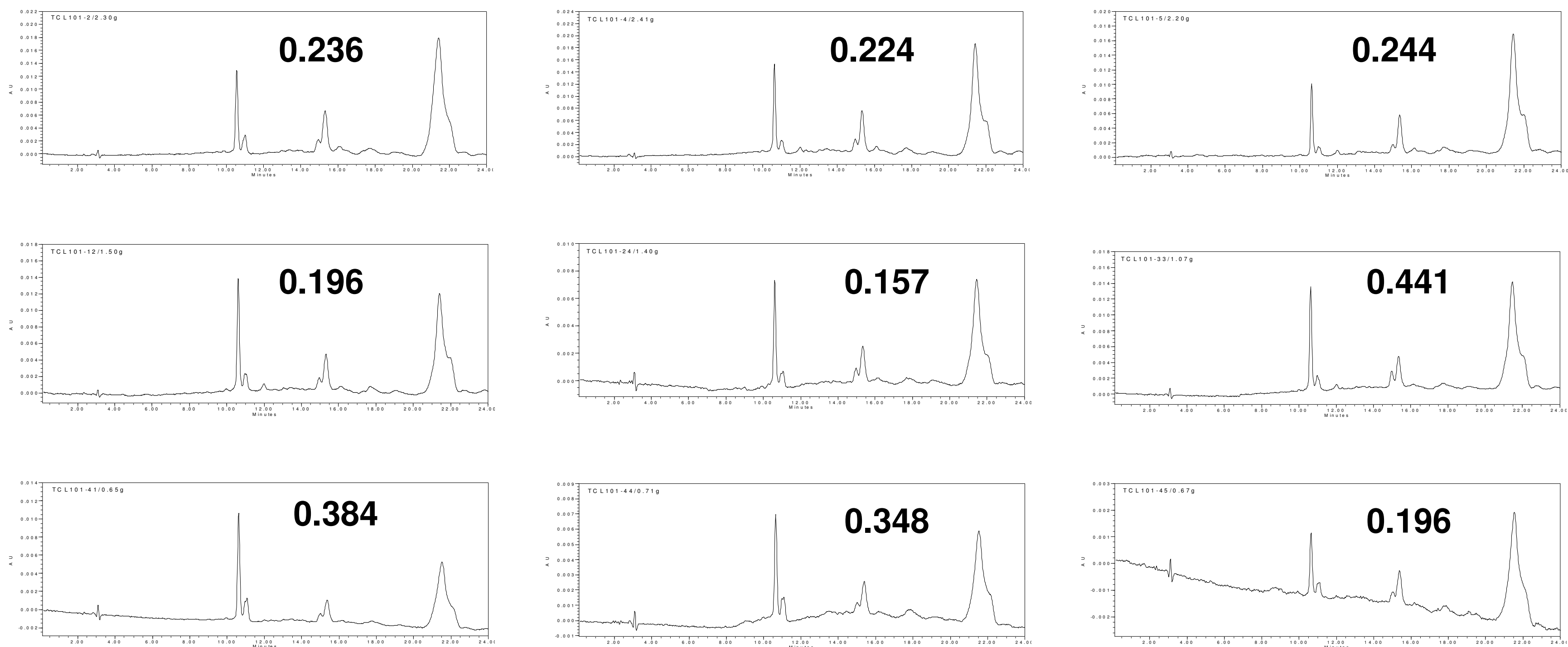


Southern blot showing integration of transgene in BR29

Southern for *psy* gene in T_1 progenies of BR29/101



Variation in the carotenoid profiles and levels of 9 selected T_1 progenies of BR29/101



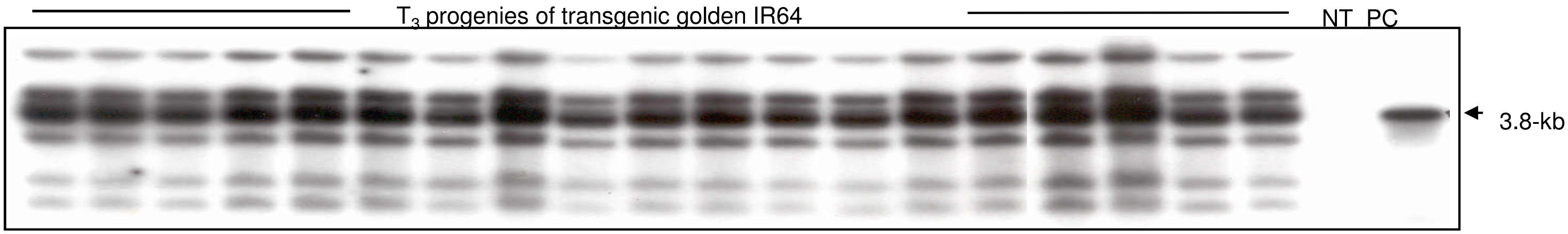


Fig. 1. Southern blot showing homozygous progenies of *Golden* indica rice (cv. IR64) with integration of a 3.8-kb fragment

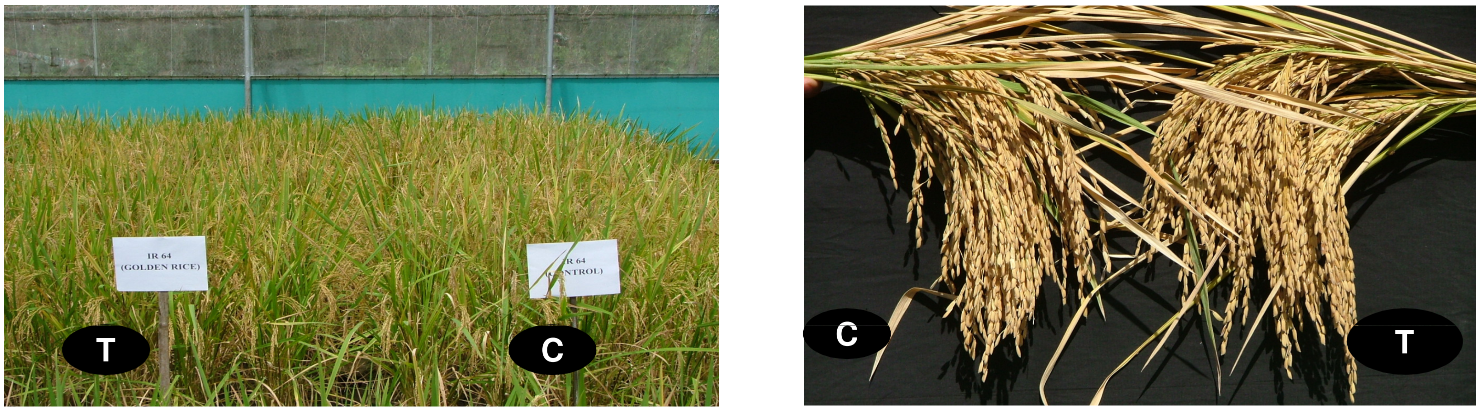


Fig. 2. Transgenic *Golden* indica rice (T) and control rice (cv. IR64; C) showing uniformity in overall phenotype (left panel) and grain filling (right panel) grown under screenhouse conditions at IRRI, Philippines.

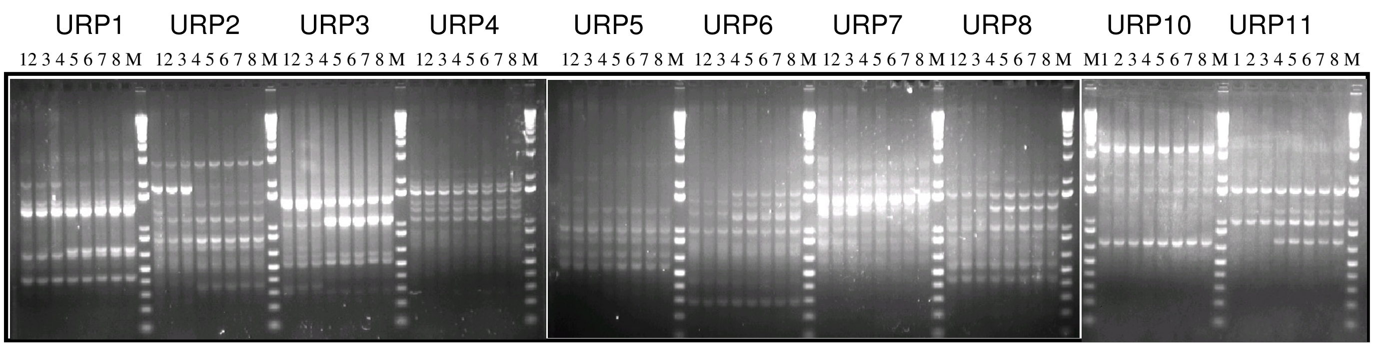


Fig. 3. Transgenic Golden indica rice of NHCD (lanes 1 and 2 in each panel) and IR64 (lanes 4, 5, 6, and 7 in each panel) showing no polymorphism with Universal rice primers (URP) vis-à-vis their respective controls (lanes 3 and 8 in each panel). M = 1 kb-plus molecular weight marker.

Agronomic performance of transgenic Golden rice (cv. IR64) vis-à-vis the IR64 control

Characters → / Treatments ↓		Plant height (cm)	No. of panicles per plant	No. of grains per panicle	No. of unfilled spikelets per panicle	Spikelet fertility (%)	1,000-grain weight (g)	Biological yield per plant (g)	Grain yield per plant (g)	Harvest index (%)
TRANSGENIC	Mean	**107.13**	**9.13**	**88.81**	**34.16**	**71.46**	**25.86**	**109.25**	**13.49**	**13.66**
	SEm±	0.745	0.358	2.460	1.364	1.078	0.168	5.953	0.661	0.610
CONTROL	Mean	**108.80**	**8.65**	**86.05**	**28.75**	**74.67**	**25.77**	**98.98**	**13.74**	**14.86**
	SEm±	1.733	0.539	5.558	3.312	2.635	0.223	9.309	1.350	1.290
F-value (transgenic vs. control)		0.950^{ns}	0.391^{ns}	0.242^{ns}	2.881^{ns}	1.627^{ns}	0.060^{ns}	0.702^{ns}	0.030^{ns}	0.770^{ns}

ns= nonsignificant at $p \leq 0.05$ (Rai et al. RGN 2004)

BR29

Table 2. Variation of carotenoid content in different progenies (selected)

Event No.	Parent No.	T0 (ug/g)	Progenies T1 (ug/g)				
			1	2	3	4	5
1	SKBR-29	1.108	2.56	2.9	3.68		
2	SKBR-59	1.108	1.73	2.21	2.2	3.05	
3	SKBR-101	-	2.04	2.63	4.21	4.46	4.49
4	SKBR-216	1.254	2.24	3.05	3.15	3.63	4.12
5	SKBR-217	-	4.56	4.98	6.43	6.59	7.55
6	SKBR-218	1.312	2.38	3.91	4.56	6.08	
7	SKBR-234	-	3.43	4.1	4.57		
8	SKBR-240	1.434	4.62	5.12	5.79		
9	SKBR-241	1.584	3.37	3.44	4.31		
10	SKBR-244	1.004	4.3	4.54	4.59	6.77	9.34
11	SK64-561	0.592	1.03	1.47	2.32	1.16	1.38
12	SK64-562	0.948	1.05	1.94			
13	SK64-560	0.748	1.08	1.32	1.25	1.08	0.76

Datta K et al 2006 Curr Sci

Essential Minerals: Iron

- Iron deficiency is the **most widespread** micronutrient deficiency worldwide.
- Approx. **30% of world population** suffers from serious nutritional problems caused by insufficient intake of iron (WHO 1992).
- It is the important **constituent of hemoglobin**, the oxygen carrying component of blood, and also a part of myoglobin that helps muscle cells to store oxygen.
- It is present in food in both inorganic (**ferric and ferrous**) and organic (**heme and nonheme**) forms. Highly bioavailable heme iron is derived primarily from animal source.

Biofortified iron rice

Screening for iron-rich rice varieties | **Mutational breeding** | **Transgenic plant strategy**

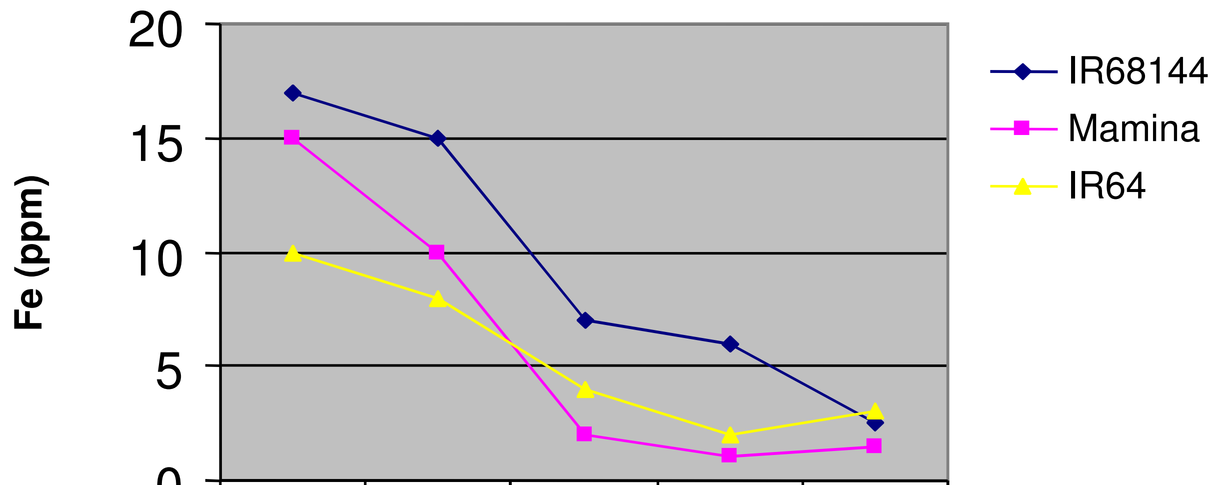
↓

1. High iron and enhanced carotenoids/beta-carotene rice
2. Reduced content of phytate in rice grains

↓

Increased bioavailabillity of Fe and Zn

Effect of different milling times in Fe content of the rice grain

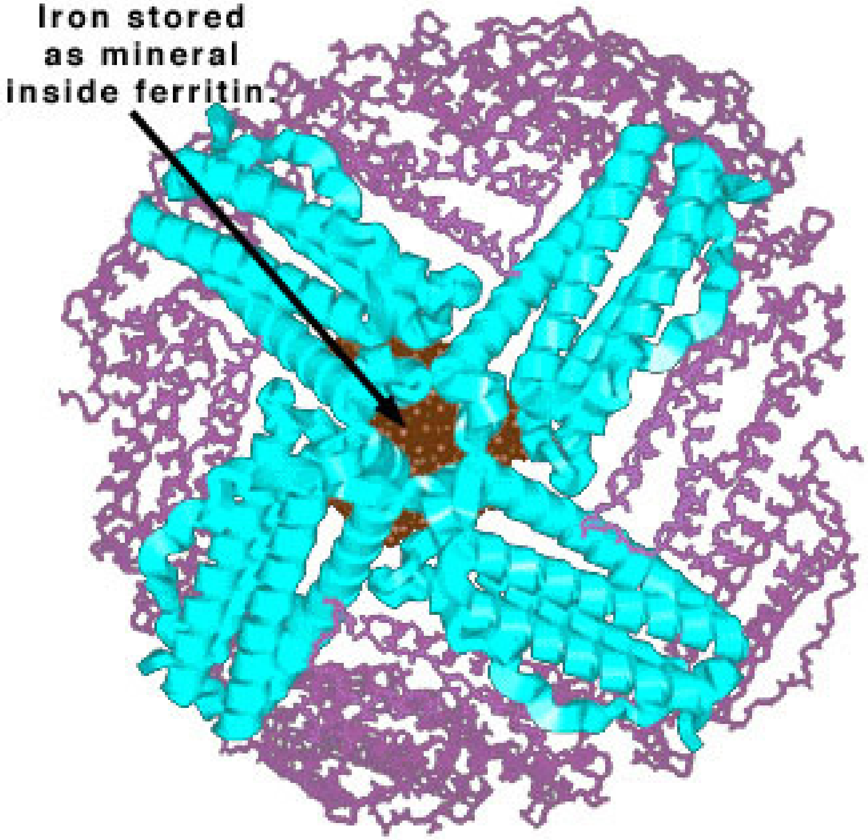


	Brown	10''	30''	1'	2''
IR68144	17	15	7	6	2.5
Mamina	15	10	2	1	1.5
IR64	10	8	4	2	3

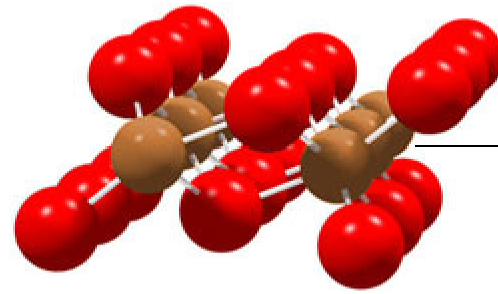
Milling time

IR68144 Mamina IR64

Ferritin : The Iron Storage Protein



Ferritin

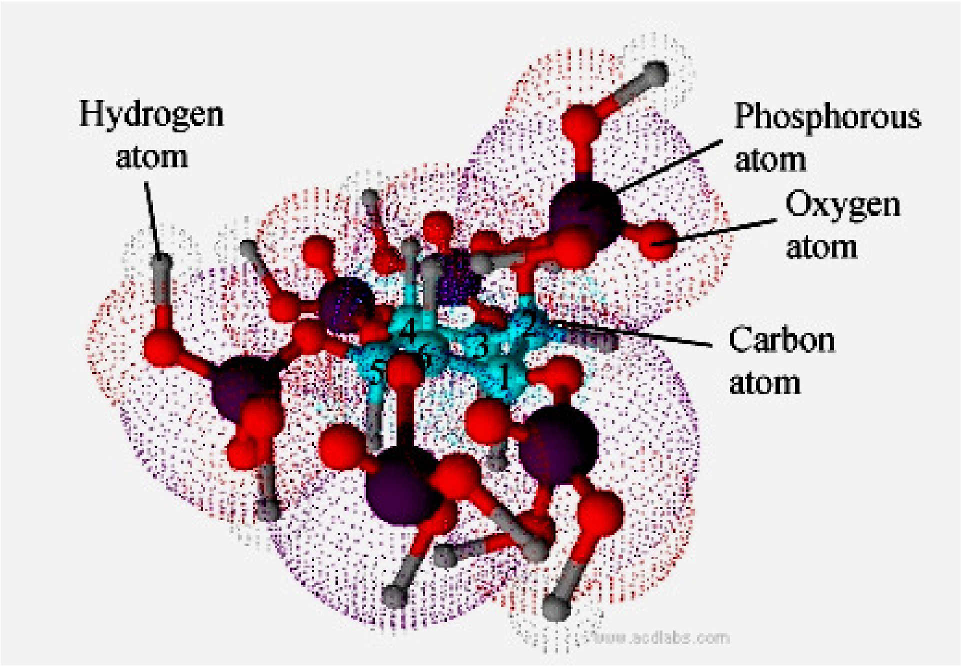


Ferrihydrite unit of ferritin $[Fe(OH)]_8[Fe\,O(H_2PO_4)]$ and FeO(OH),repeated in a specific pattern in ferritin molecule

Iron is stored within protein shell of spherical ferritin molecule, which can store about 4500 iron(III) ions and regulate the levels of available iron in the body by releasing iron in a controlled fashion

Phytic acid

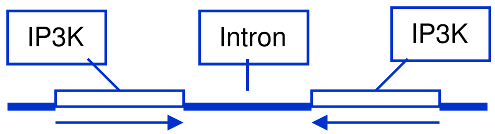
- Phytic acid, Myo-inositol (1,2,3,4,5,6) hexa*kis*phosphate is primary storage compound of phosphorus.
- Negatively charged phosphate in PA strongly binds to metallic cations of CA, Fe, K, Mg, Mn, and Zn making them insoluble and thus unavailable as nutritional factors.
- Phytate mainly accumulate in protein storage vacuoles and located in aleurone layer.



Phytic acid

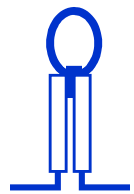
Silencing of $InsP_3$-kinase by RNAi

DNA



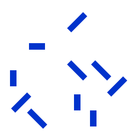
↓

Hairpin forming RNA



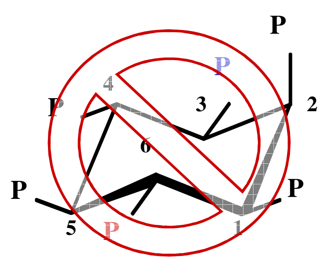
↓

Sequence specific breakdown of endogenous IP3K RNA



↓

Silencing of IP3K



Advantages:

- Effective silencing ≈ 90-100% (Wesley et al. 2001)
- Specific down regulation of single gene
- Only small part of gene sequence required

Vector: pGreen II delta BUM/HUM

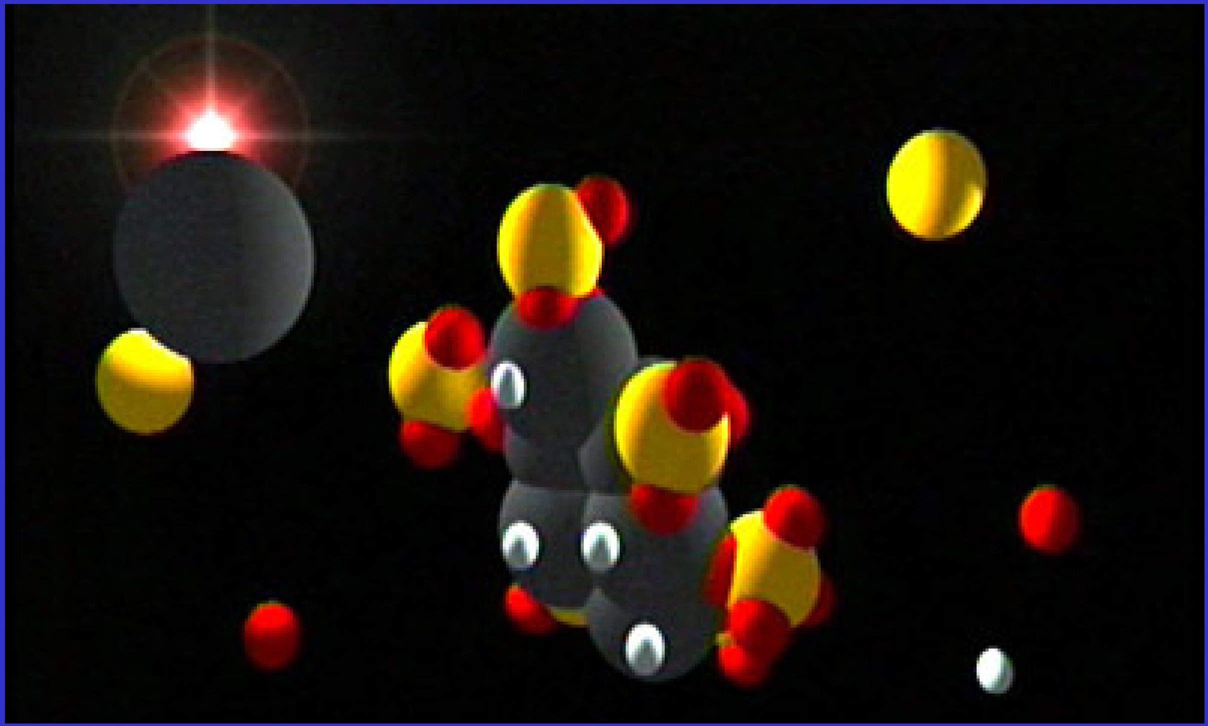
Promotor: Actin and Ubiquitin (selection)

Selection: Basta / Hygromycin

Transformation:

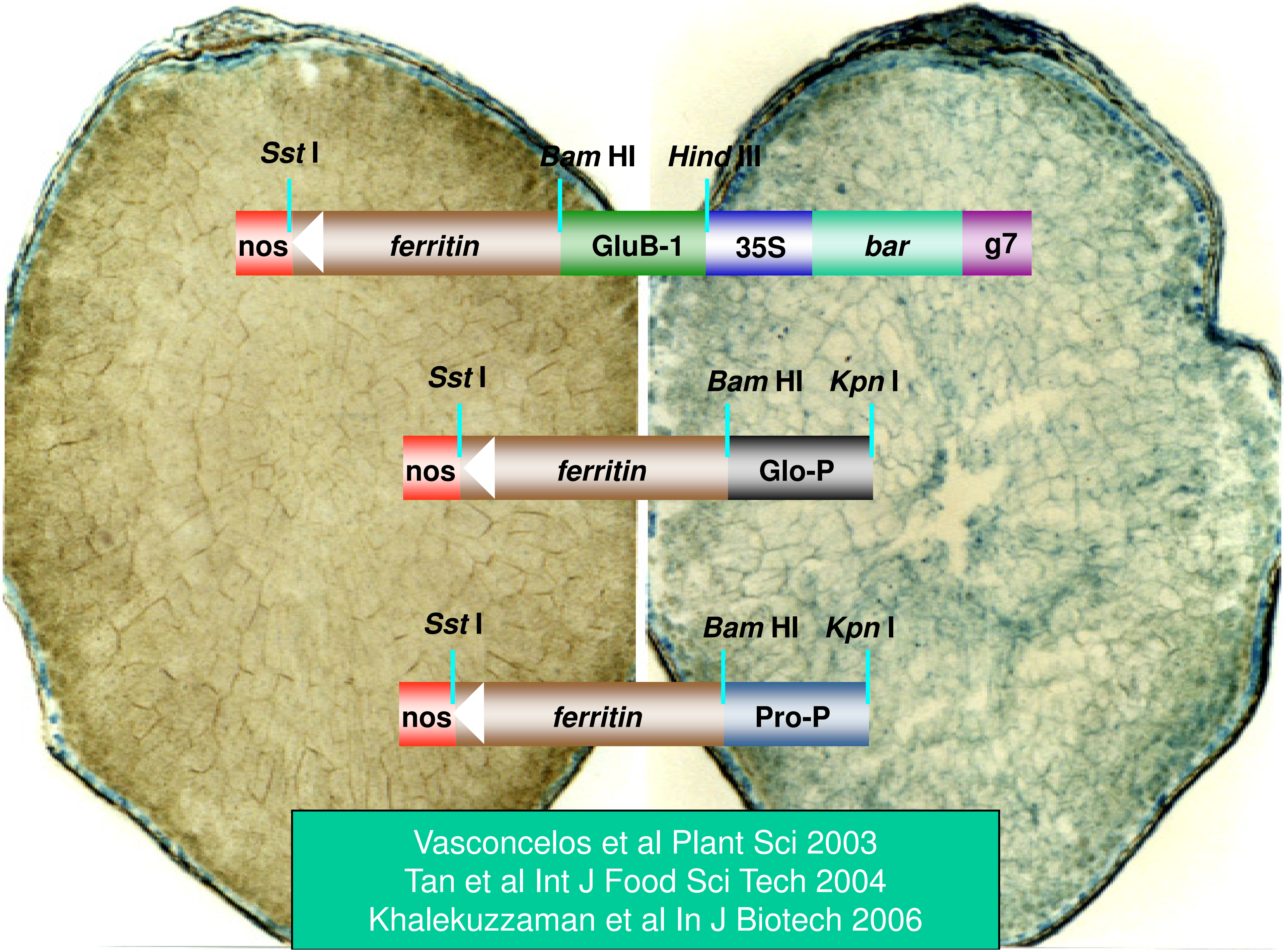
Biolistic and Agrobacterium

Low Phytate canola/rice meal

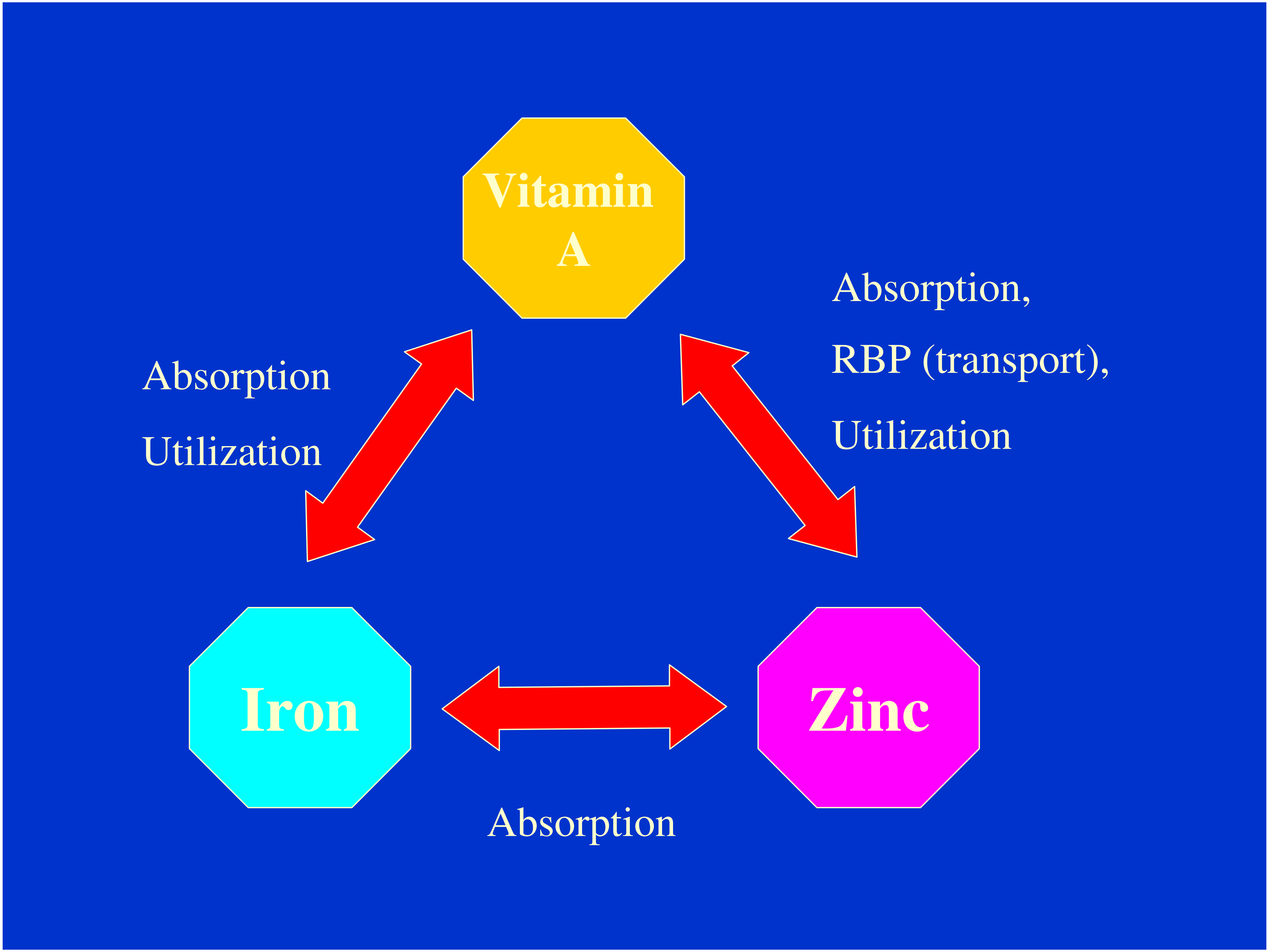


Phytate molecule (shown here) strongly binds to proteins and essential minerals. This prevents their absorption by animals, making canola meal unattractive as a feed stock. However, by genetically modifying canola, NRC researchers reduced the amount of phytate in the meal by about 50-per cent. **Phytate-reduced canola meal could be an excellent animal feed, including for aquaculture.**

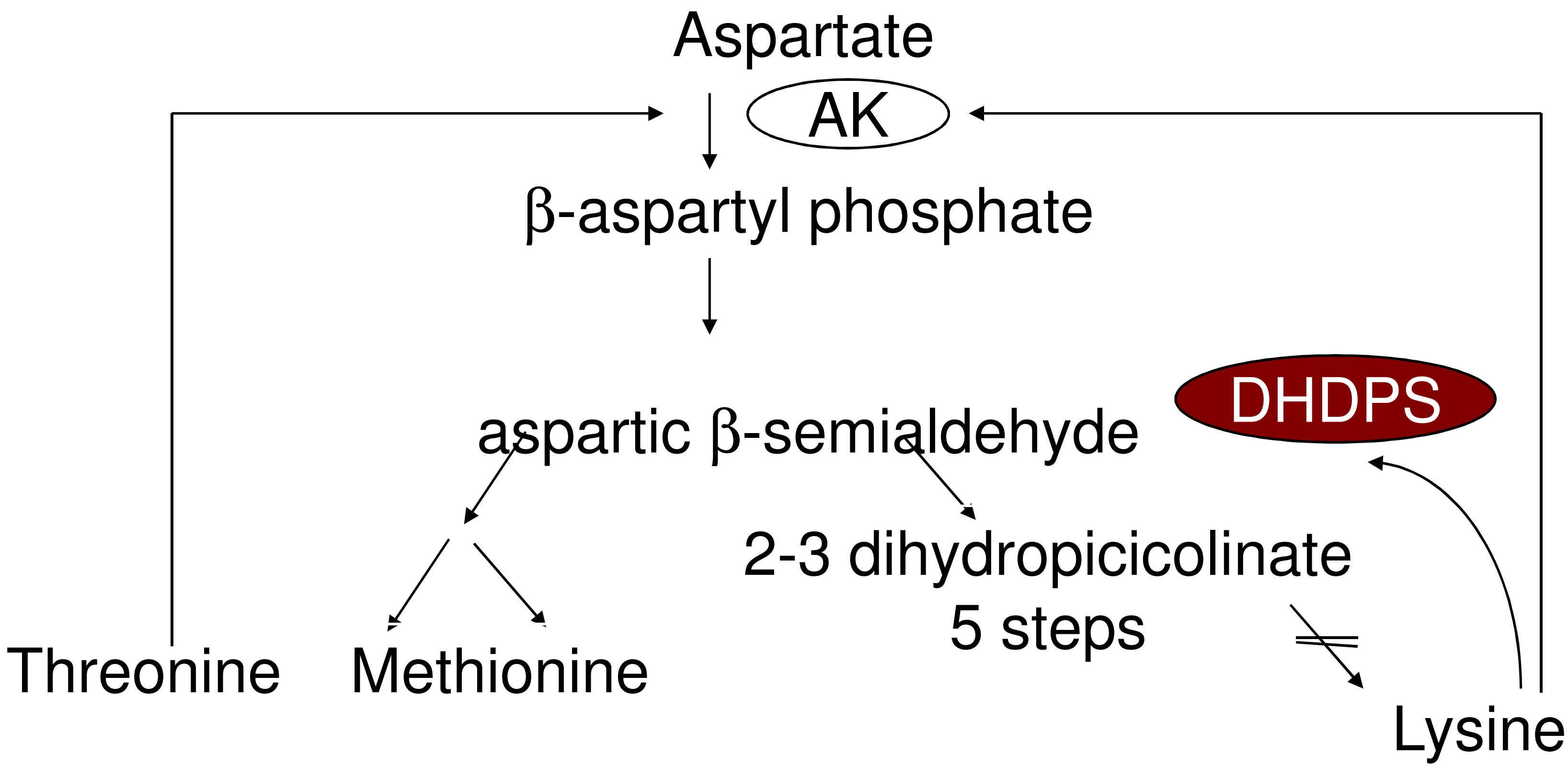
NRC Plant Biotechnology, Canada & (Agri. & Agri Food, Canada)



Vasconcelos et al Plant Sci 2003
Tan et al Int J Food Sci Tech 2004
Khalekuzzaman et al In J Biotech 2006

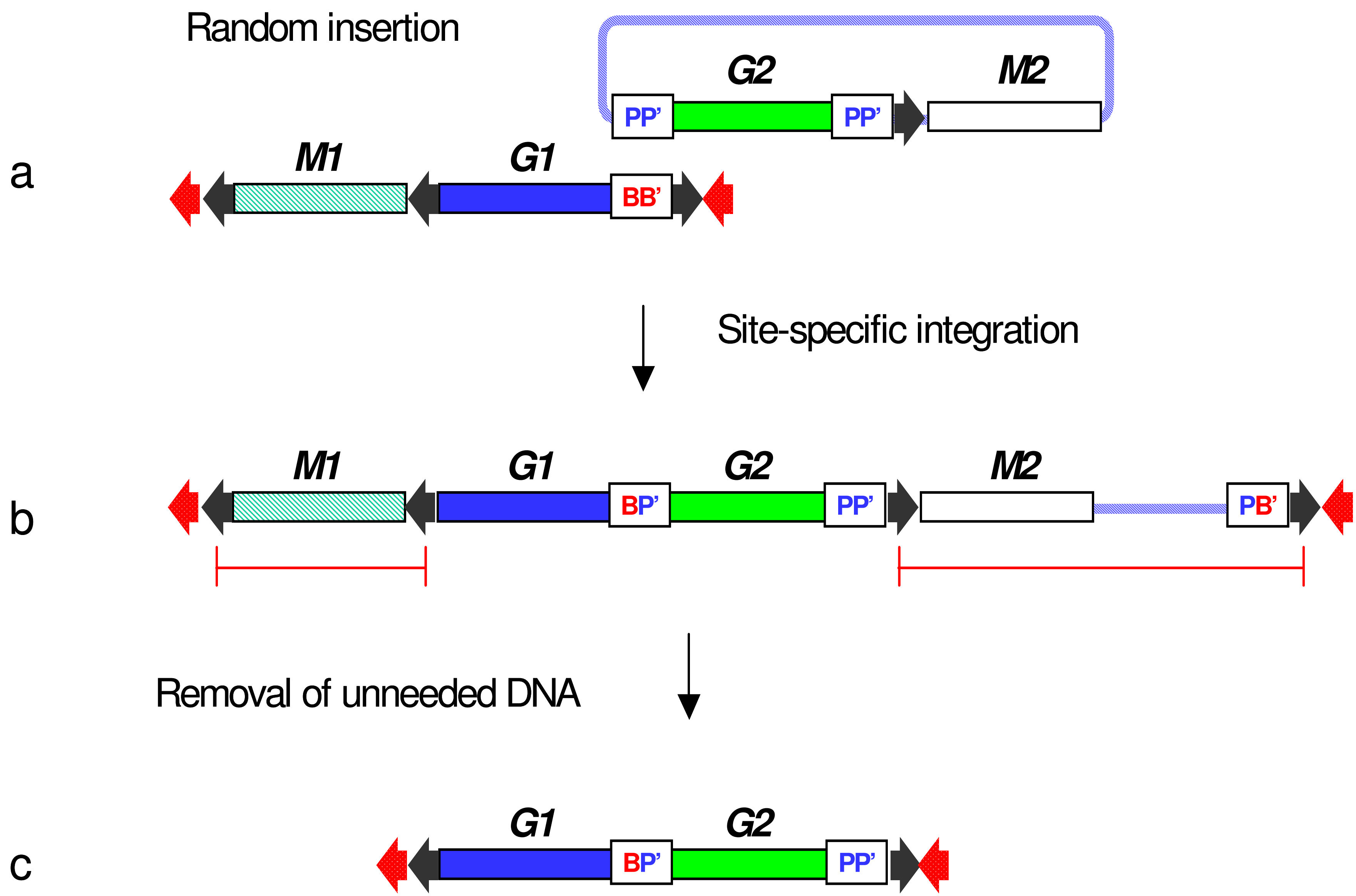

Vitamin A
Absorption,
RBP (transport),
Utilization
Absorption
Utilization
Iron
Zinc
Absorption

The Aspartate-Family Biosynthetic Pathway



GM BioFoods (selected)

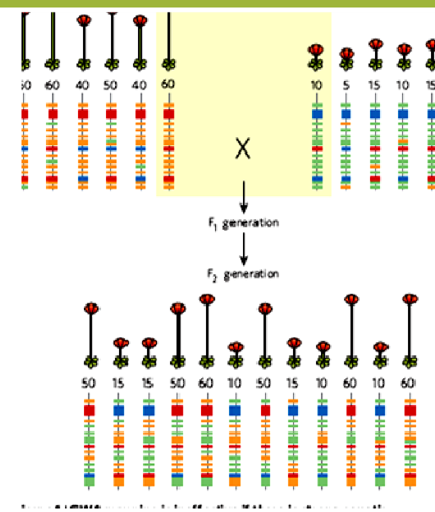
Food crop	Trait	Genetic element	Source	Reference
Canola	**Improved fatty acids** **(high oleic & low linolenic acid)**	Fatty acid desaturase	**Mutation Conv. breeding**	Pioneer
Maize	**Enhanced lysine level**	*dapA (DHDPS)*	***Corynebacterium glutamicum*** **(rice, maize)**	DuPont
Potato	**Enhanced Amino acid**	*AMA1*	***Amaranthus*** **(rice, bacteria)**	Chakra arty et al 1999
Potato	**Pro-VitA**	*Or1*	**Cauliflower**	Lopez et al 2008
Soybean	**Improved fatty acid (high oleic acid)**	δ12 Fatty acid desaturase	***Glycine max***	DuPont
Rice	**Pro-VitA**	*psy, crt1, lcy*	**Rice, maize, daffodills**	Ye et al. 2000 Datta et al. 2003,
Rice	**High iron**	Ferritin	***Glycine max***	Goto et al. 1999 Vasconcelos et al 2003,MS Swaminathan Foundation, 2006



Random insertion
G2
M2
PP'
PP'
a
M1
G1
BB'
Site-specific integration
b
M1
G1
G2
M2
BP'
PP'
PB'
Removal of unneeded DNA
c
G1
G2
BP'
PP'

INSIGHT PROGRESS

NATURE|Vol 456|11 December|doi:10.1038/nature07629



Next-generation genetics in plants

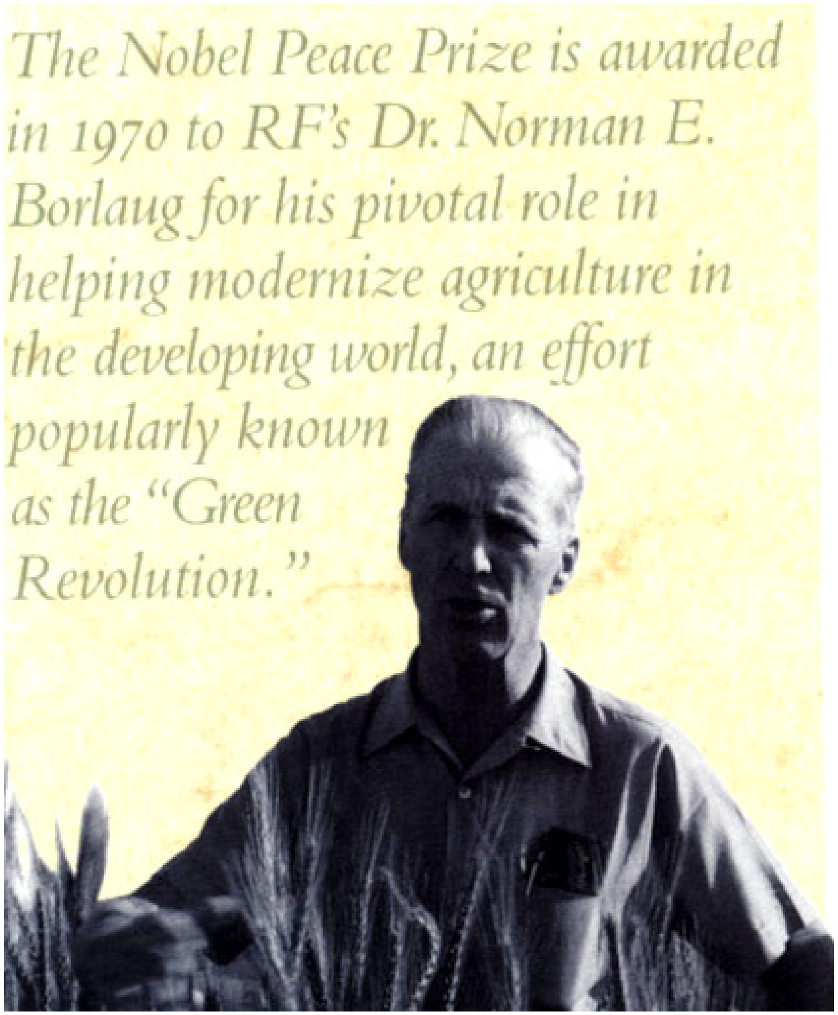
Magnus Nordborg[1] & Detlef Weigel[2]

Natural variation presents one of the fundamental challenges of modern biology. Soon, the genome sequences of thousands of individuals will be known for each of several species. But how does the genotypic variation that will be observed among these individuals translate into phenotypic variation? Plants are in many ways ideal for addressing this question, and resources that are unmatched, except in humans, have now been developed.

Technology is neutral: Availability and use is subject to……..

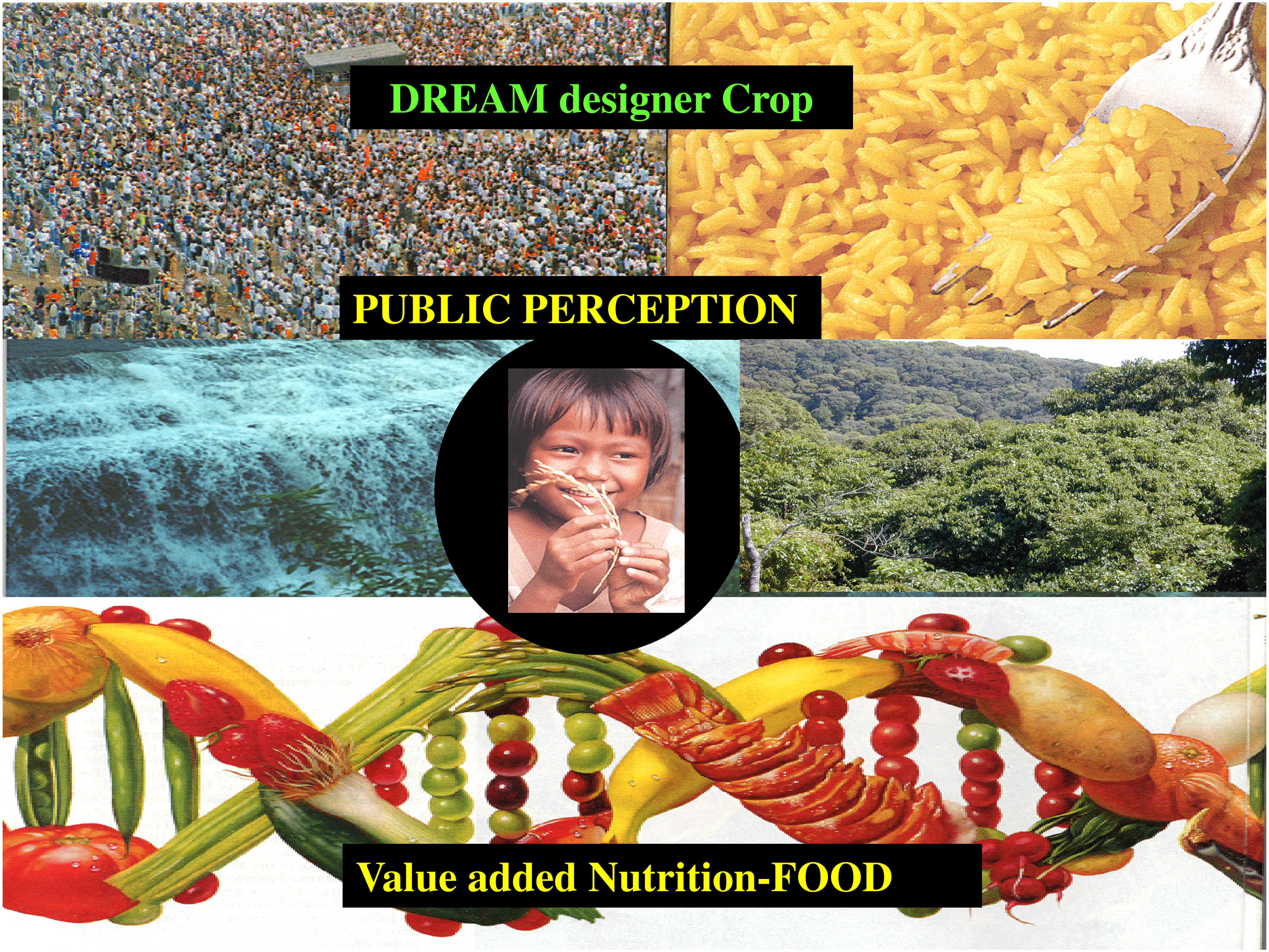
- Nutrition crops (Golden canola, Golden rice, Carotenoids enriched maize, vitamin E maize, high iron rice, Low phytate crops etc.) available.

- Too much regulatory restrictions + Political/Govt. will and the policy of technology donors need serious attention to make the products available to the people who need them most.



Green revolution saved famine in Asia

Molecular breeding for Nutrition food may help in reducing malnutrition provided FTO is in place


DREAM designer Crop
PUBLIC PERCEPTION
Value added Nutrition-FOOD