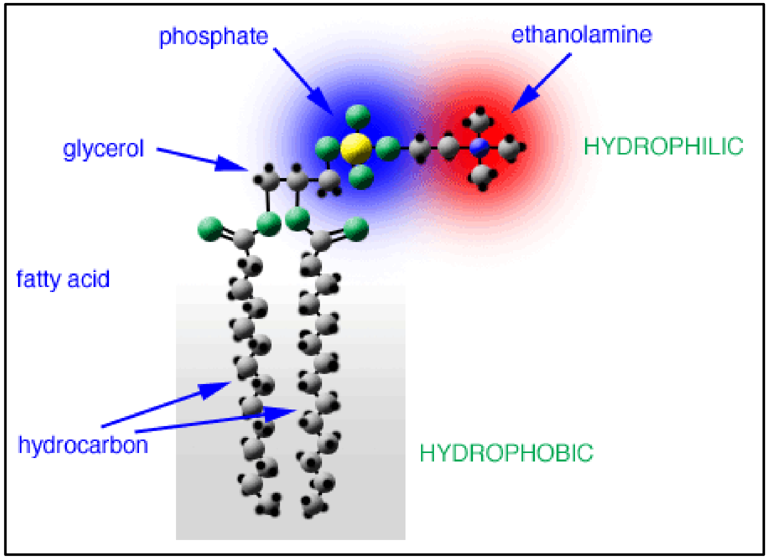
Improving Nutrient-Use Efficiency in Crop Plants

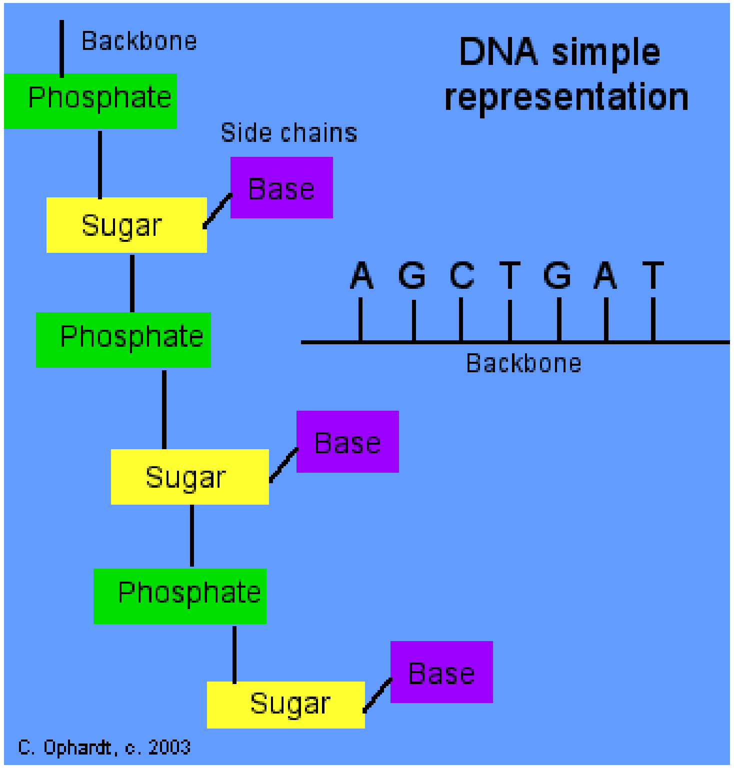
Assoc. Prof. Dr. Ahmad M. Manschadi

Phosphorus (P)

- Phosphate is not reduced in plants (unlike N and S)
  - Inorganic phosphate (P_i)
  - Esterified through a hydroxyl group to a C chain (C—O—P)
  - Pyrophosphate bond (energy-rich): P—P (ATP)
  - Diester bond (C—P—C): forming bridging groups connecting units to macromolecules

- **Structural element**
  - In DNA and RNA: forming a bridge between ribonucleoside units
  - Phospholipids of biomembranes

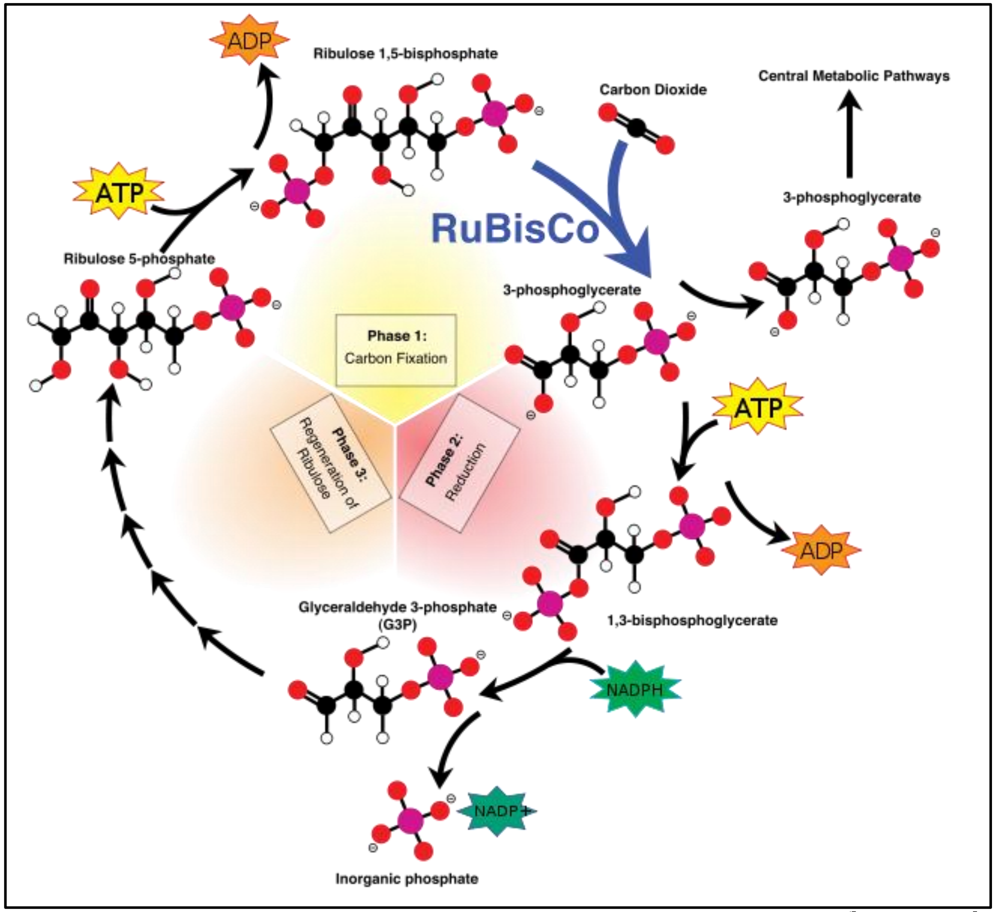




- **Role in energy transfer**
- Phosphate esters (C—P) and energy-rich phosphates (P—P) represent the "**metabolic machinery**" of cells
- Most P-esters are intermediates in metabolic pathways of **biosynthesis** and **degradation**
- **ATP** provides energy for e.g. starch biosynthesis and ion uptake
- **ATPases**: enzymes that catalyse the decomposition of ATP into ADP and a free phosphate
- ATPases activity affected by **Mg, Ca, K**
- **Phosphorylation**: addition of a phosphate group to a protein or other organic molecule (**kinases**)
- **Dephosphorylation** (**phosphatases**)

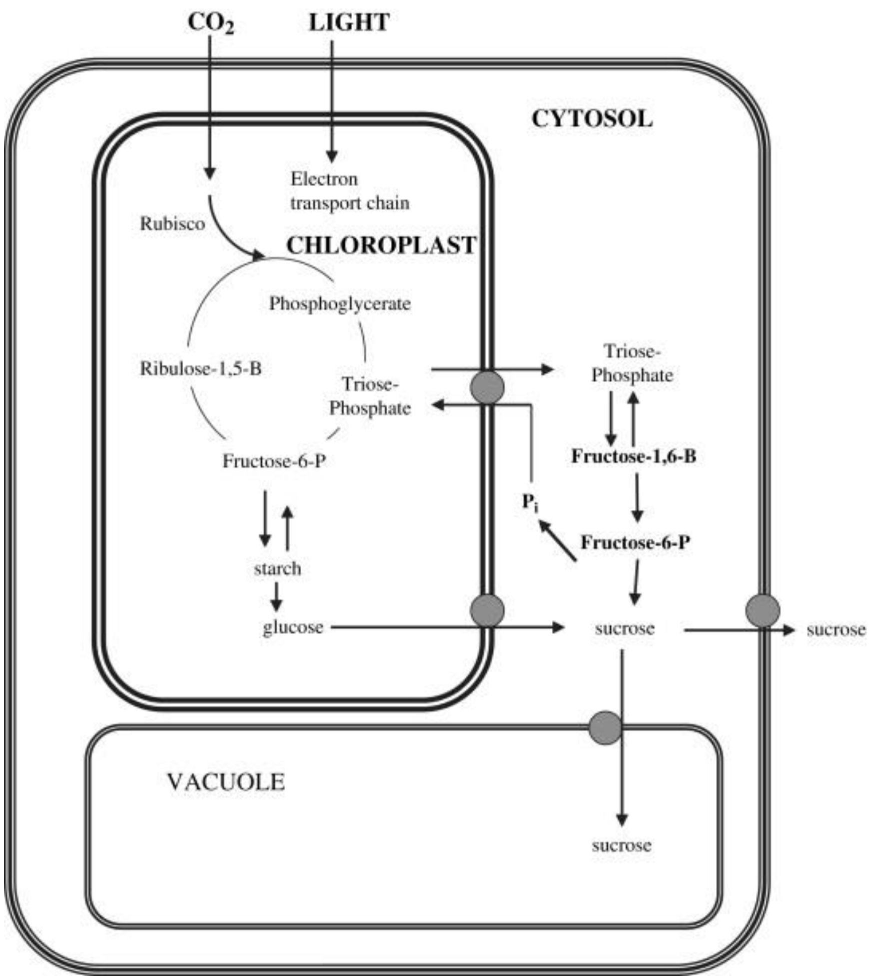
  → turning many protein enzymes on (activated) and off (deactivated)
- **Signalling transduction** – phosphorylation increases the activity of PEP carboxylase
  → the enzyme becomes less sensitive to negative feedback control by high malate concentrations

- **Role of inorganic phosphate (P_i)**
- **Compartmentation of P_i**– storage in vacuoles ("**nonmetabolic pool**") → release in cytoplasm → enzymes activation → fruit ripening (respiratory burst)
- P_i in cytoplasm and chloroplasts - "**metabolic pool**"
- Large demand of P_i for phosphorylated intermediates of photosynthesis



(http://en.wikipedia.org/wiki/File:Calvin-cycle4.svg)

- **P_i regulates carbon partitioning between chloroplasts and cytosol**
  - **Photosynthesis**: C fixation through Calvin cycle in the chloroplast → export of triose phosphate (triose-P) to the cytosol → conversion of triose-P to sucrose → release of Pi → available to allow further export of triose-P from the chloroplast



(Kaschuk et al. 2009)

- **P_i regulates carbon partitioning between chloroplasts and cytosol**
  - **4 trios-P + 3 H_2O → 1 sucrose + 4 P_i**
  - Reduced sucrose synthesis in the cytoplasm → decreased export of triose-P from the chloroplast → activation of starch-synthesizing enzyme ADPG → starch synthesis
  - Role of source-sink relationship

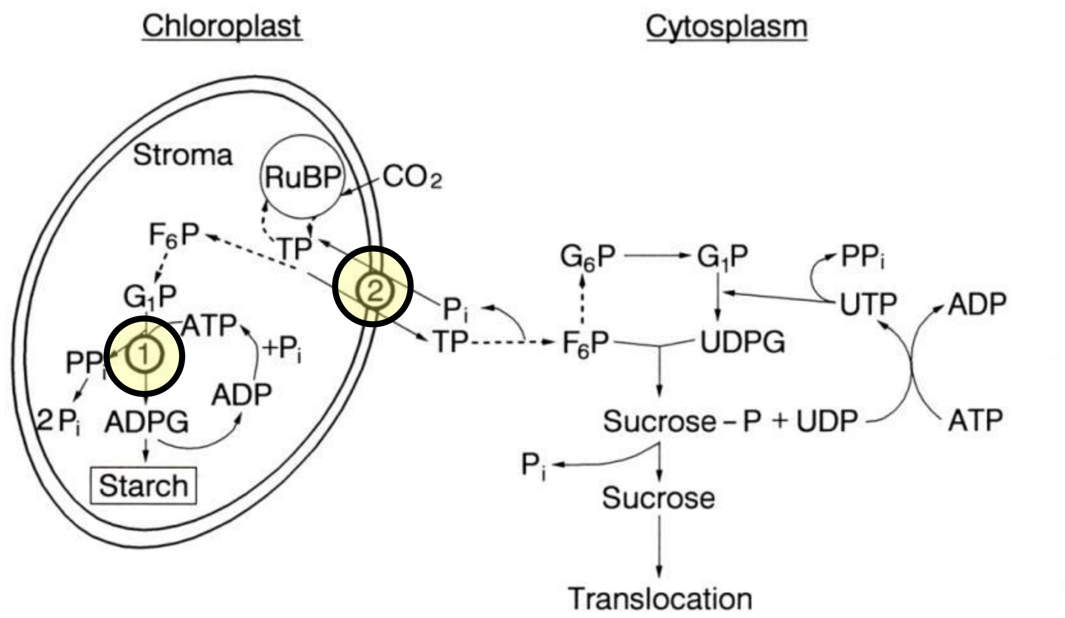
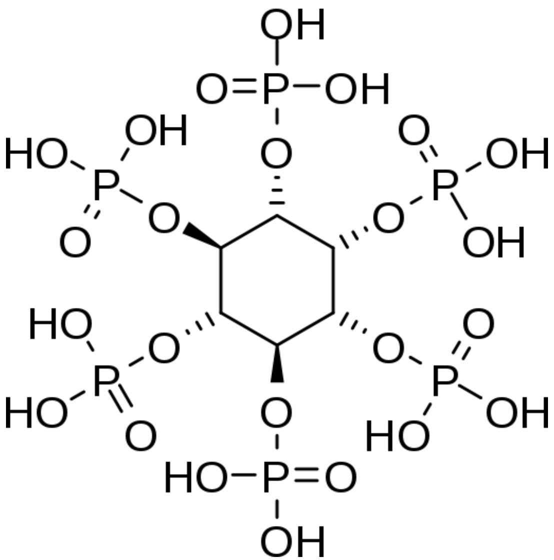


Fig. 8.20 Involvement and regulatory role of phosphate in starch synthesis and carbohydrate transport in a leaf cell. (1) ADP-glucose pyrophosphorylase: regulates the rate of starch synthesis; inhibited by P_i and stimulated by PGA. (2) Phosphate translocator: regulates the release of photosynthates from chloroplasts; enhanced by P_i. TP, Triosephosphate (glyceraldehyde-3-phosphate, GAP; dihydroxyacetone phosphate; DHAP); F_6P, fructose 6-phosphate; G_6P, glucose 6-phosphate. (Based on Walker, 1980.)

(Marschner 1995)

- **P_i regulates carbon partitioning between chloroplasts and cytosol – Summary**
  - **P_i deprivation**
    - does not affect photosynthetic electron transport
    - reduces photosynthesis through the limitation of RuBP regeneration activity
    - RuBP regeneration limited by the supply of ATP and by increased partitioning of sugar phosphates to starch
    - affects leaf area most and photosynthesis to a lesser extent
    - diminishes carbon export more than the rate of photosynthesis
    - carbon accumulates in leaves of Pi-deprived plants
    - P_i-deprivation effects on photosynthesis and carbon partitioning are reversible
    - sink strength imposes the most important regulatory role on photosynthesis during phosphate deficiency

- **Other storage forms of phosphate**
- **Polyphosphates** – linear polymers of P_i
  - Energy storage and control of P_i level in metabolic pool
  - Formation in hyphae of mycorrhizal fungi
- **Phytate** – storage form in grains and seeds
  - Salts of phytic acid (myo-inositol)
  - Makes up 50% (legumes seeds) and 60-70% (cereal grains) of total P
  - Not digestible to humans and nonruminant animals
  - High affinity of phytic acid to K, Mg, Ca, Zn, Fe, and other heavy metals



- **Phytate** – storage form in grains and seeds
  - Grain-filling: increase in phytate synthesis – decrease in Pi
  - Seed germination: degradation of phytate by **phytases** → release of Pi, K, Mg
  - Incorporation of Pi into phospholipids (membrane synthesis)
  - Increase in inorganic P – onset of respiration, phosphorylation
  - Increase in DNA/RNA – cell division and net protein synthesis

Table 8.16
Changes in Phosphorus Fractions of Rice Seeds during Germination[a]

Duration of germination (h)	Phosphorus fraction (mg P g^{-1} dry wt)				
	Phytate	Lipid	Inorganic	Ester	RNA + DNA
0	2.67	0.43	0.24	0.078	0.058
24	1.48	1.19	0.64	0.102	0.048
48	1.06	1.54	0.89	0.110	0.077
72	0.80	1.71	0.86	0.124	0.116

[a]From Mukherji *et al*. (1971).

(Marschner 1995)

- Phytate and human health

- Phytate hydrolysis and dephosphorylation during food processing
  - Germination: best results after 6 – 10 days
  - Soaking: phytate is water soluble → discarding the soak water
    optimal conditions for phytate dephosphorylation: 45 - 65 °C and pH=5.0 - 6.0
  - Cooking: phytate is heat-stable, discarding the cooking water
  - Fermentation: Phytase present in cereal flour; microorganisms (yeast & lactic acid bacteria) during beard making
  - Addition of isolated phytases during food processing:
    - Phytate-degrading activity depends on raw material used, manufacturing process, source and amount of phytase
    - Temperature, pH,

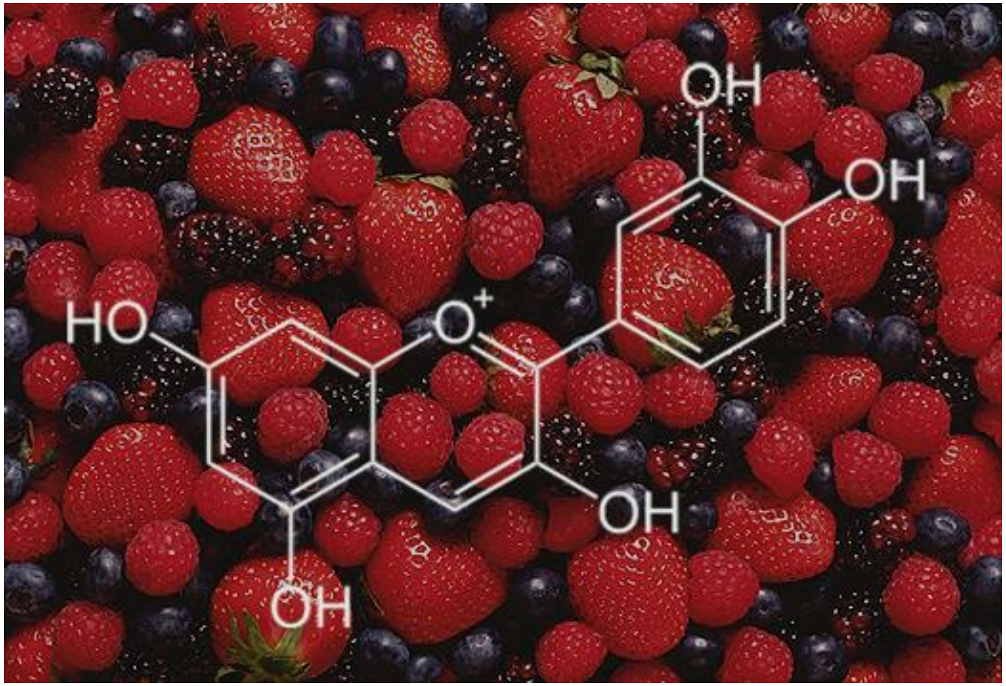
(Greiner & Konietzny 2006)

P deficiency

- Young plants: dwarfed and thin with dark green/blue-green leaves
- Older leaves are affected first
- Leaf margins, veins and stems show purple tints which may spread over the whole leaf blade

P deficiency

- No effect on chlorophyll synthesis → higher chlorophyll a conc. per unit leaf area
- In legumes: leaf chlorosis due to reduced N fixation induced by P deficiency
- Higher production of NADPH/H^+ and ATP (photosynthesis light reaction) than demand for the reduction of CO_2
- Transfer of H^+ to other molecules such to yield flavanones → dihydroflavonols → anthocyanins
  - **Anthocyanins** (flavonoids family): water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH
  - Autumn leaf colour; cold weather in autumn; drought stress

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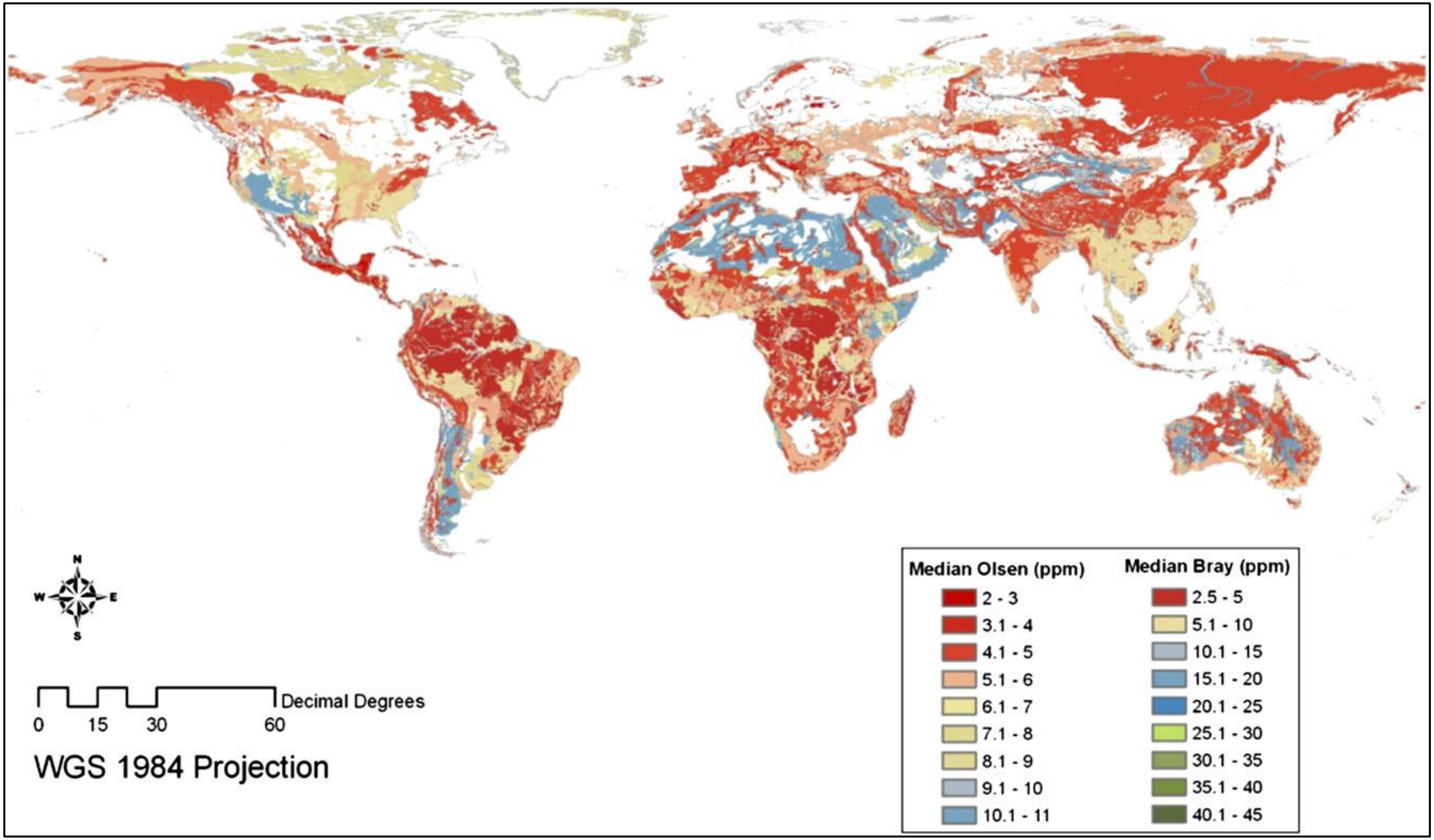
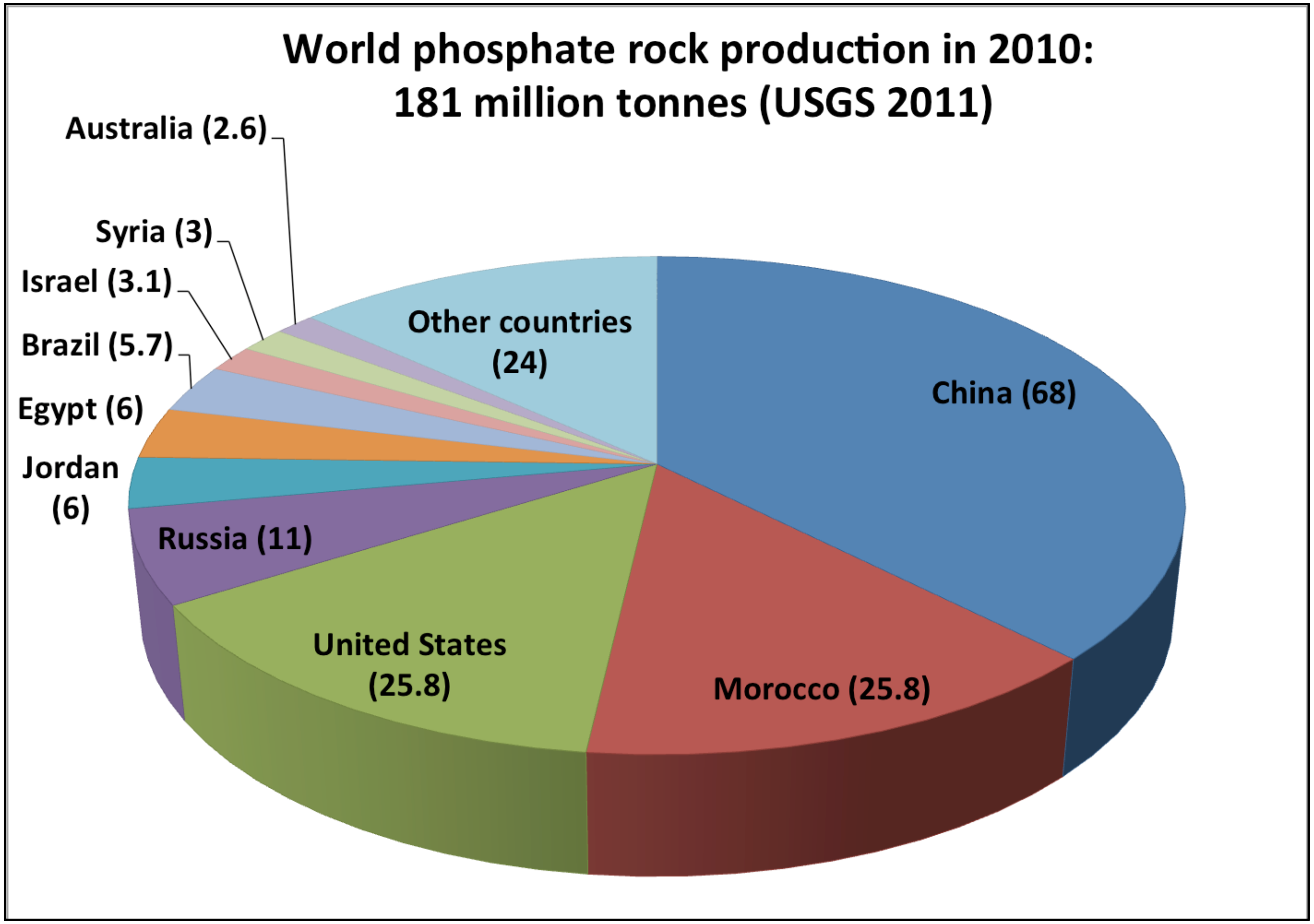
Phosphorus

- Over half of the global agricultural land is characterised by low P availability

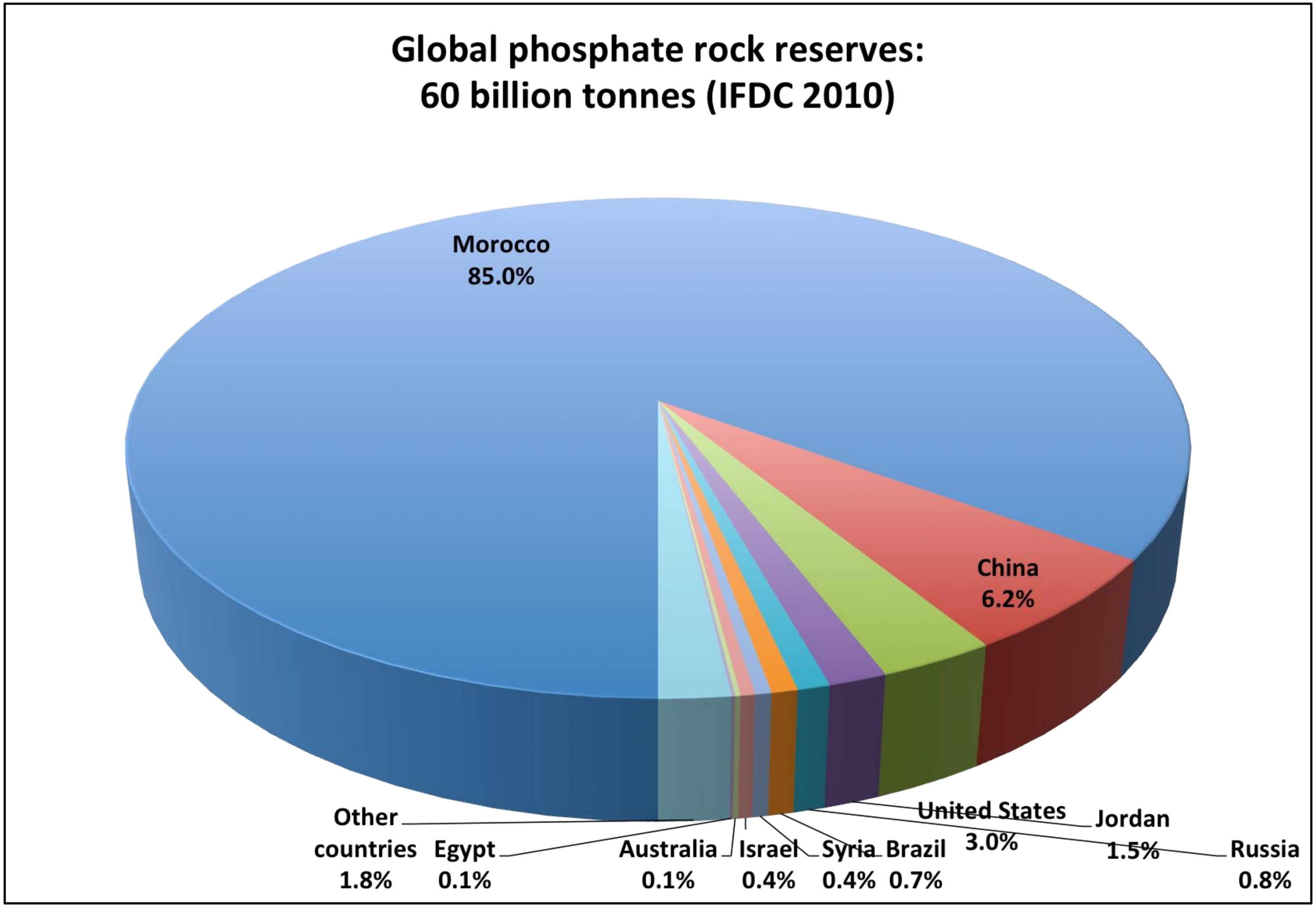


(Jaramillo-Velastagui 2011)

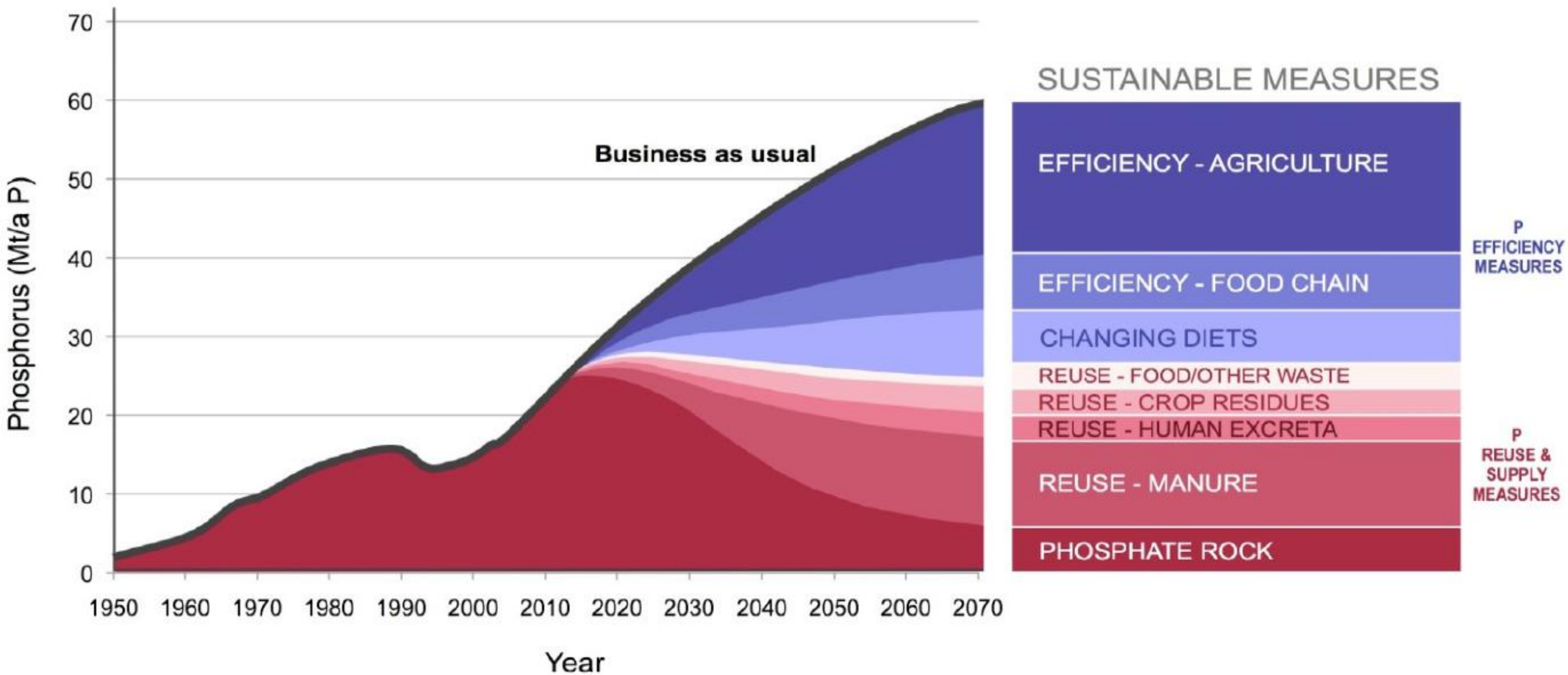
- Phosphorus (P): essential and unsubstitutable inorganic nutrient
- **Non-renewable resource**: decreasing global P reserves; increasing P-fertiliser prices
- Two-third of the world PR production: 4 countries (China, Morocco, United States, and Russia)



- Global commercial PR reserves: will be depleted in 50-400 years
- Concerns about global P security

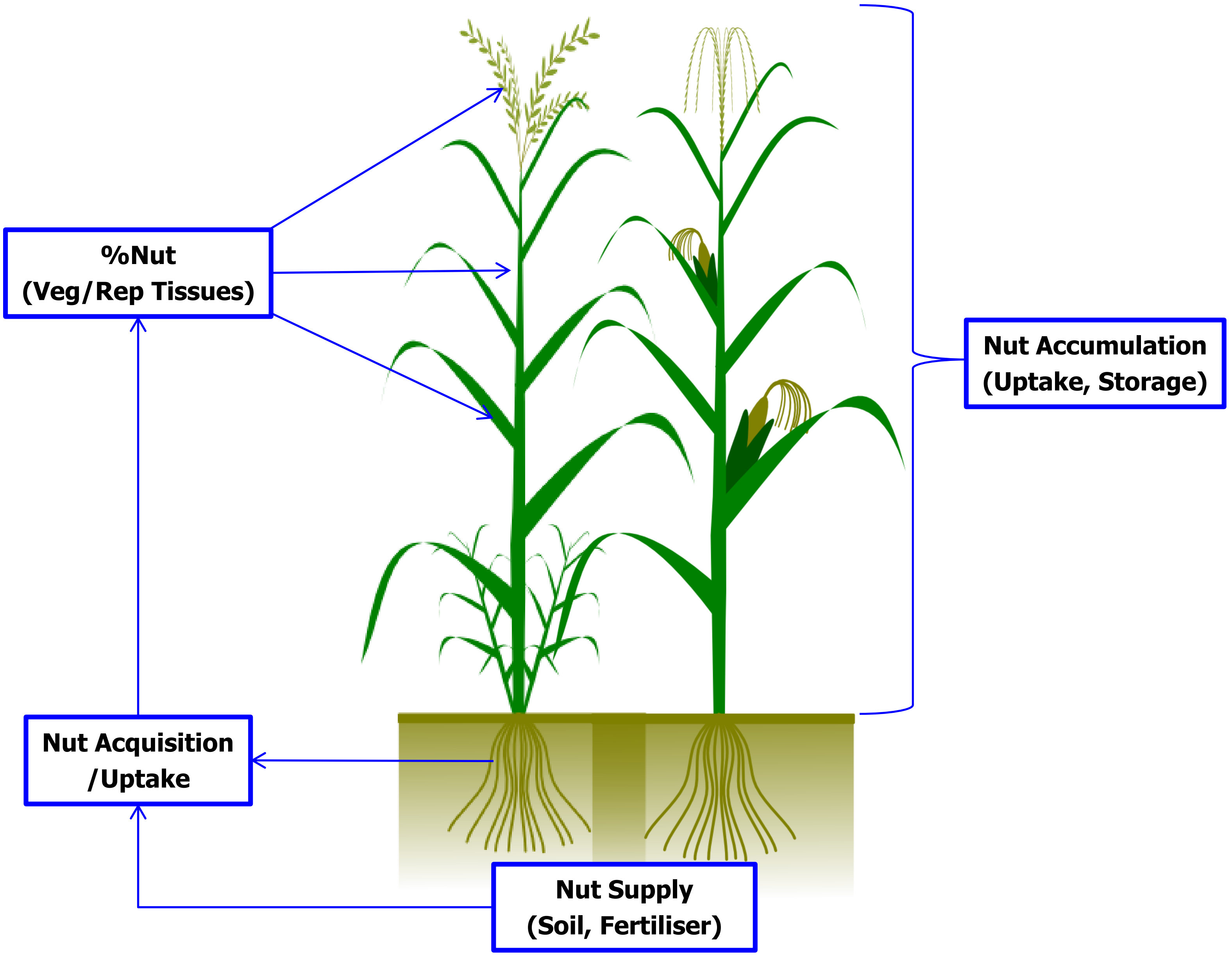


- Sustainable future pathway:
  - High P recovery and reuse from the food chain
  - Large reduction in P demand: **Increasing efficiency in agricultural use**
    Reducing losses in the food chain; changing diets



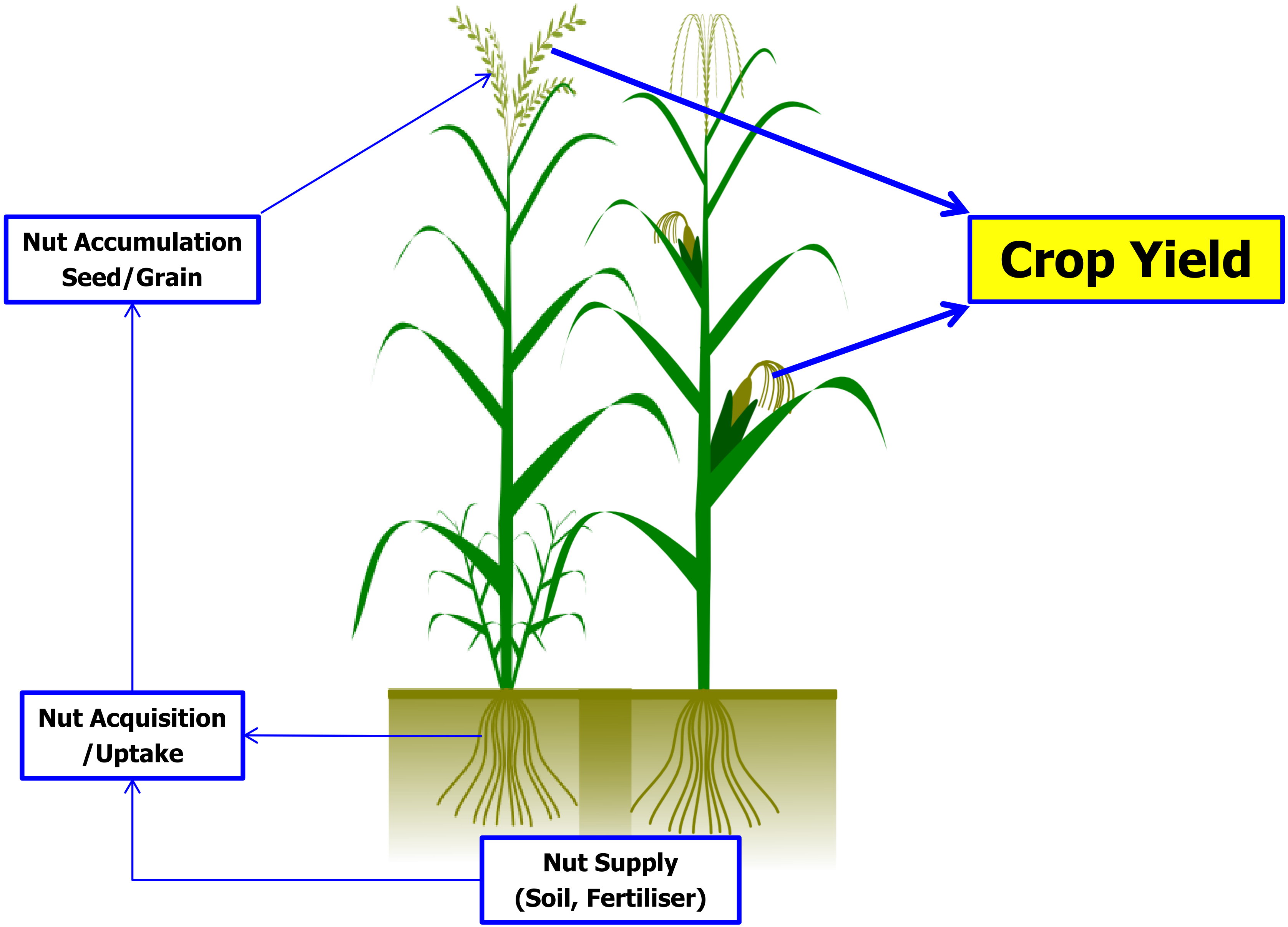
(Cordell et al. 2011, Chemosphere)

Nutrient-use efficiency



- **Nutrient-use efficiency (NutUE):**

  Crop yield per unit of nutrient supply from soil and fertiliser



- **Nutrient-use efficiency (NutUE)**:

  Crop yield per unit of nutrient supply from soil and fertiliser

- Product of two subcomponents:
  - **Nutrient-acquisition efficiency (NutAE)** –

    Total nutrient in the above-ground plant organs at maturity per unit of nutrient supply
  - **Nutrient-utilisation efficiency (NutUtE)** –

    Crop seed yield per unit of nutrient taken up

    The internal efficiency with which the absorbed nutrient is utilised to produce yield

$$NutUE = NutAE \times NutUtE$$

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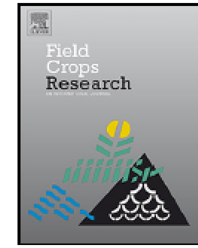


Developing phosphorus-efficient crop varieties—An interdisciplinary research framework

Ahmad M. Manschadi[a,*], Hans-Peter Kaul[a], Johann Vollmann[b], Josef Eitzinger[c], Walter Wenzel[d]

[a] *University of Natural Resources and Life Sciences, Vienna, Department of Crop Sciences, Division of Agronomy, Konrad Lorenz Str. 24, 3430 Tulln, Austria*
[b] *University of Natural Resources and Life Sciences, Vienna, Department of Crop Sciences, Division of Plant Breeding, Konrad Lorenz Str. 24, 3430 Tulln, Austria*
[c] *University of Natural Resources and Life Sciences, Vienna, Department of Water, Atmosphere and Environment, Institute of Meteorology, Peter-Jordan-Str. 82, 1190 Vienna, Austria*
[d] *University of Natural Resources and Life Sciences, Vienna, Department of Forest and Soil Sciences, Institute of Soil Research, Konrad Lorenz Str. 24, 3430 Tulln, Austria*

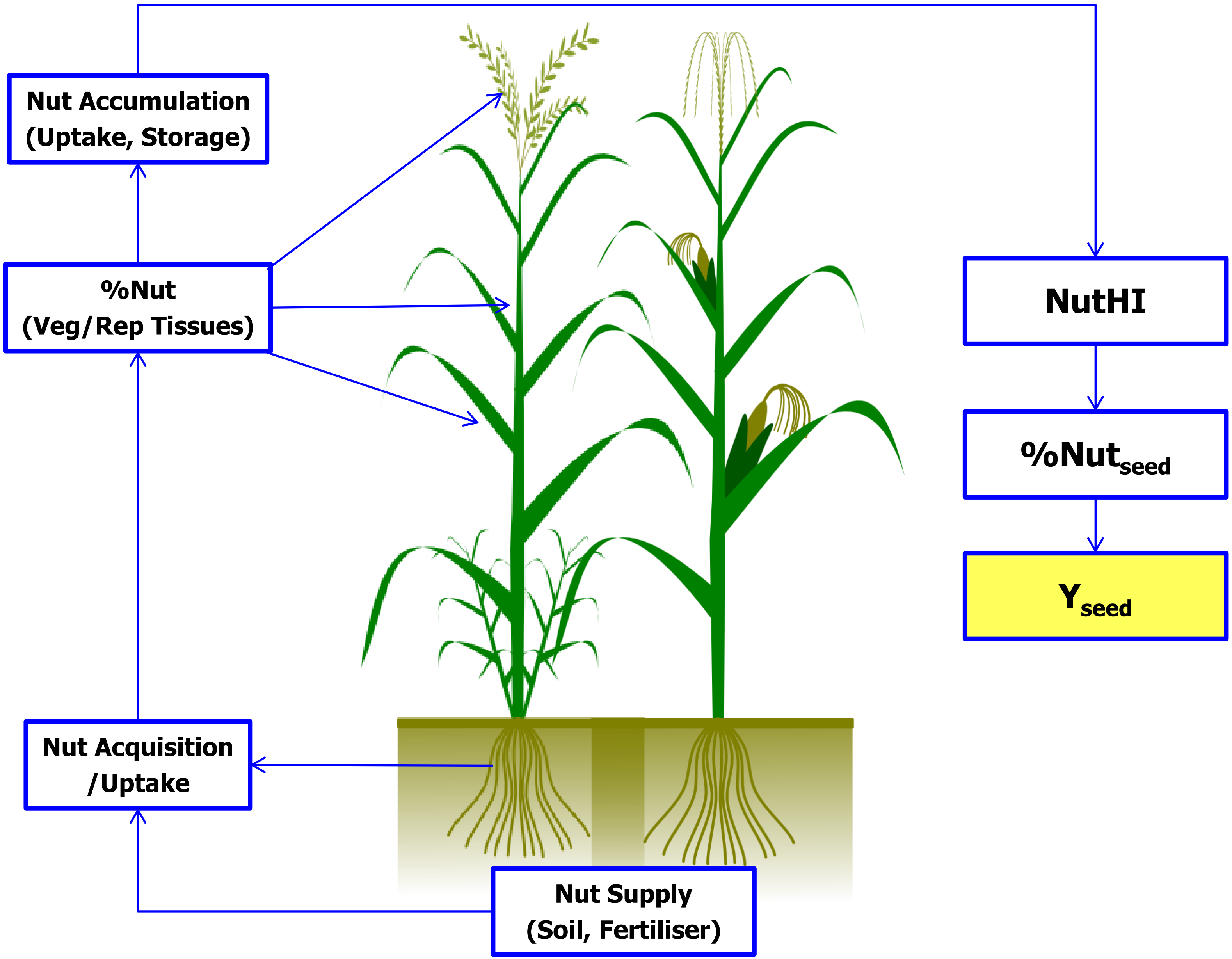
Maximum nutrient-limited crop yield (Y_{seed}):

$$Y_{seed} = Nut_{accum} \times \frac{NutHI}{\%Nut_{seed}}$$

- **Nut_{accum}**: total accumulated nutrient in the plant (kg Nut ha^{-1})
- **NutHI**: fraction of total accumulated nutrient in the plant that is allocated to the seed
- **$\%Nut_{seed}$**: seed nutrient concentration

(Sinclair & Rufty 2012)

Nutrient-use efficiency (NutUE)



Nitrogen-use efficiency (NUE)

$$Y_{seed} = Nut_{accum} \times \frac{NutHI}{\%Nut_{seed}}$$

- **Reducing $\%Nut_{seed}$:**
  - Constrained by commercial demands for high seed protein content
  - Special end-user markets
  - Improvements in NUE of modern crop varieties due to
    - **Reduction in grain %N (consequence of increased HI & yield)**
    - **Increase in nitrogen harvest index (NHI)**

Nitrogen-use efficiency (NUE)

- **Reduction in grain %N (consequence of increased HI & yield)**

Comprehensive review of 100 papers reporting maize yield, N fertiliser, N uptake, planting density:

"**Old Era**" (1940-1990) & "**New Era**" (1991-2011)

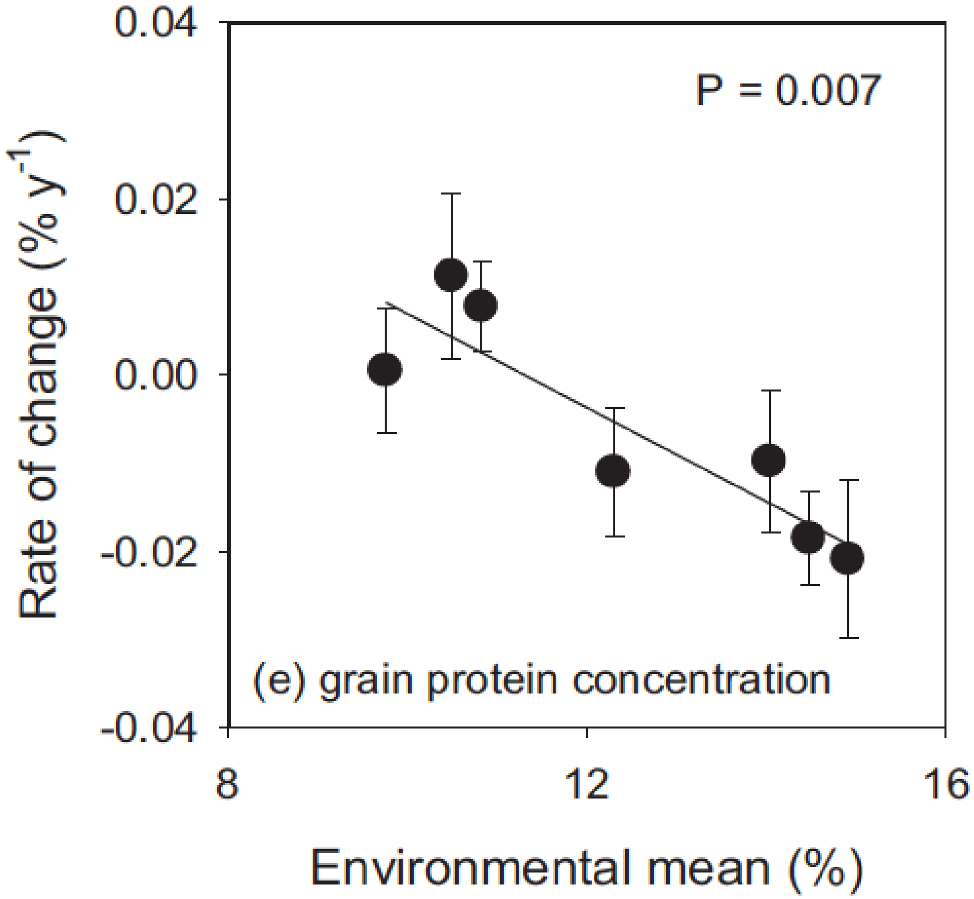
Variable	Old Era	New Era
Grain yield (kg ha^{-1})	7200	9000
Total BM (kg ha^{-1})	14000	18900
HI	47.6	49.8
Total N uptake (kg ha^{-1})	152	170
Grain N uptake (kg ha^{-1})	98.3	112
NHI	**63.1**	**63.8**
Plant %N	1.03	0.93
Stover %N	0.77	0.69
Grain %N	**1.33**	**1.20**
NUE (kg grain kg^{-1} N applied)	**19.1**	**22.7**

(Ciampitti & Vyn 2012)

Nitrogen-use efficiency (NUE)

- **Reduction in grain %N (consequence of increased HI & yield)**

Australian wheat varieties: Released between 1958 and 2007



(Sadras & Lawson 2013)

Nitrogen-use efficiency (NUE)

- Reduction in grain %N (consequence of increased HI & yield)
- **Increase in nitrogen harvest index (NHI)**

Crop species	NHI	Reference
Spring barley (European)	0.65 – 0.85	Bingham et a. (2012)
Durum wheat (Italian)	0.78 – 0.80	Giunta et al. (2007)
Wheat (Australian)	0.63 – 0.74	Sadras & Lawson (2013)
Winter wheat (European)	0.69 – 0.98	Barraclough et al. (2010)
Winter wheat (UK & French cv.)	0.73 – 0.83	Gaju et al. (2011)
Maize (studies from 1940 to 2011)	0.64 – 0.84	Ciampitti & Vyn (2012)

- **Nitrogen-use efficiency (NUE)**
  - Reduction in grain %N (consequence of increased HI & yield)
  - Increase in nitrogen harvest index (NHI)

$$Y_{seed} = Nut_{accum} \times \frac{NutHI}{\%Nut_{seed}}$$

- **Major challenge: increase in Nut_{accum}**
  - Enhancing the N storage capacity of vegetative tissues (Rubisco enzyme)
  - Improving the N uptake capacity of roots during veg. growth
  - Extending the N uptake capacity of roots into the reproductive phase
  - Delaying leaf senescence

- **Law of conservation of matter:**

High yield of high quality grain requires a high input and uptake of N

Phosphorus-use efficiency (PUE)

- **Reduction in grain %P**
- **Increase in phosphorus harvest index (PHI)**

$$Y_{seed} = Nut_{accum} \times \frac{NutHI}{\%Nut_{seed}}$$

Reducing seed P concentration

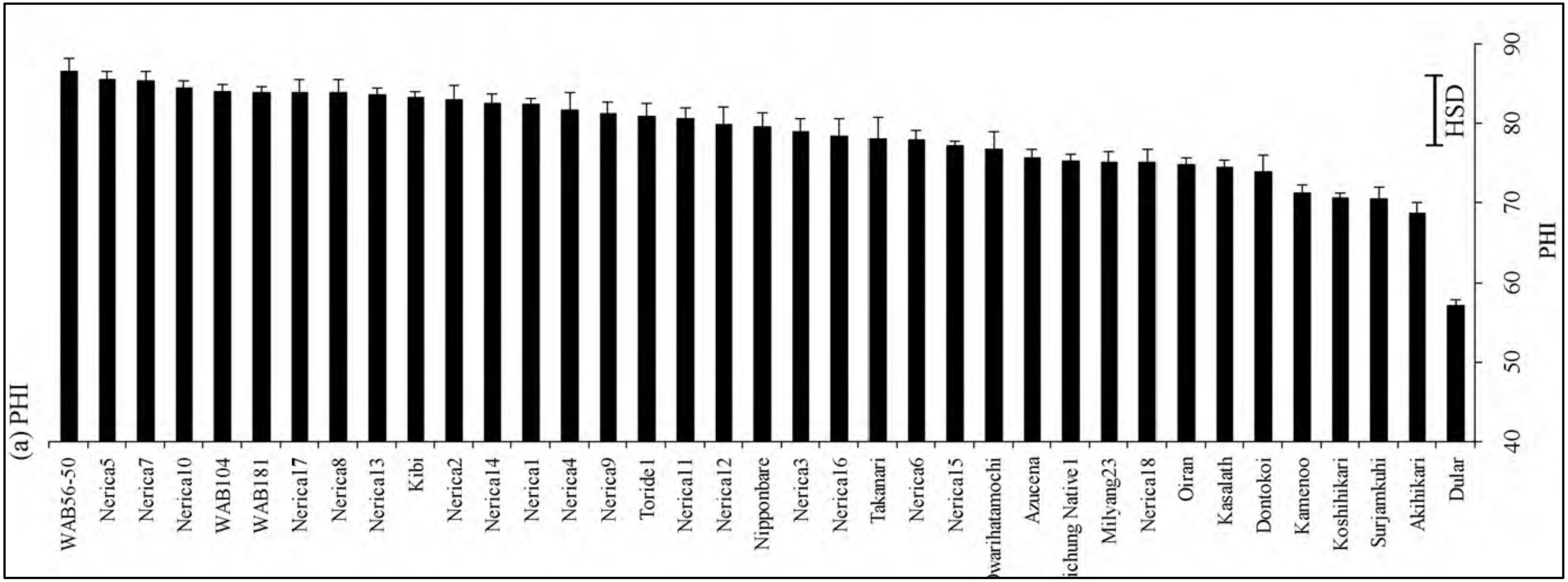
- Phytic acid: major organic storage form of P (75% of total seed P)
- Phytate salts (K, Mg, Ca, Fe, Zn): non-available for humans and monogastric animal
- Excretion of phytate: environmental hazard and waste management problem
- Breeding low phytic acid crop genotypes?
- **Ratio of P to other nutrients; seed germination; human health???**

(Manschadi et al. 2014)

Phosphorus-use efficiency (PUE)

- Reduction in grain %P
- **Increase in phosphorus harvest index (PHI)**

Rice: genotypic variability in PHI (57 – 87)

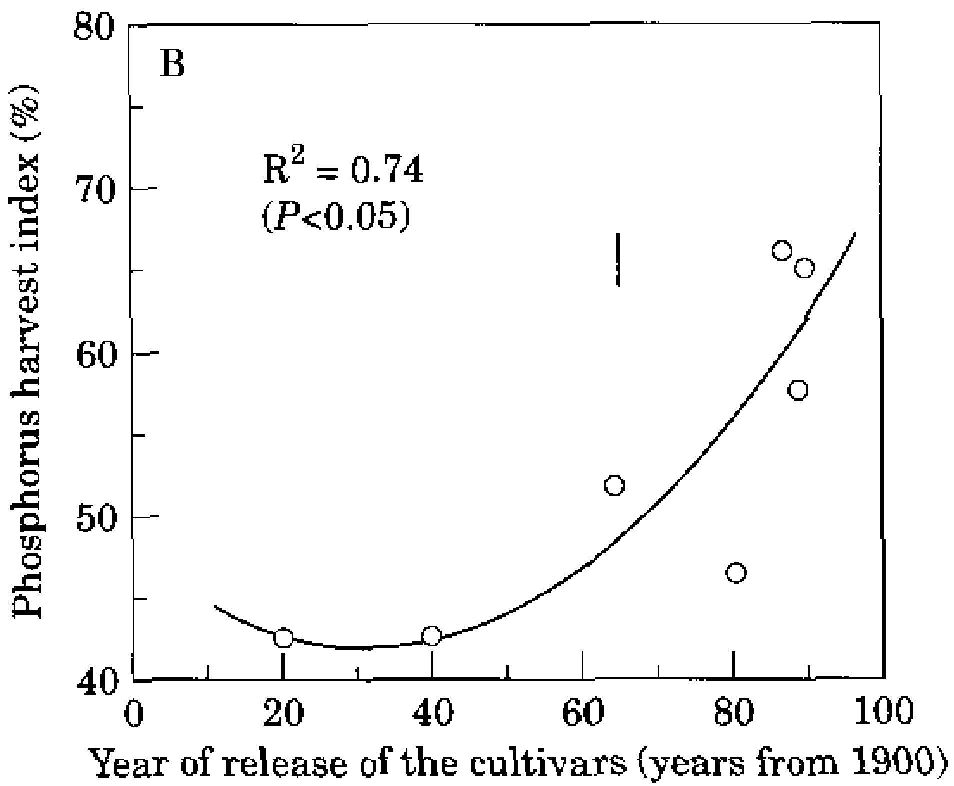


(Rose et al. 2010)

Phosphorus-use efficiency (PUE)

- Reduction in grain %P
- **Increase in phosphorus harvest index (PHI)**

Wheat: Trend in PHI since 1900



(Calderini et al. 1995)

- **Phosphorus-use efficiency (PUE)**
  - Reduction in grain %P
  - Increase in phosphorus harvest index (PHI)
  - **Increasing P accumulation (*Nut_{accum}*)**

- Upper limit for P acquisition and storage (per-unit-area basis):

  amount of vegetative mass x tissue P concentration

Phosphorus-use efficiency (PUE)

- Reduction in grain %P
- Increase in phosphorus harvest index (PHI)
- **Increasing P accumulation (Nut_{accum})**

- **Management**: plant population density, water/nutrients supply, length of growth period
- **Genetics**: photosynthetic capacity, partitioning of photosynthates, P concentrations
  - Uptake of P fertiliser: 15 – 30% in the year of application
  - P-adaptive traits:
    - **R:S biomass ratio**
    - **Root architecture and root hairs**
    - **Production and secretion of phosphatases and organic acids**
    - **Symbiotic associations with mycorrhizal fungi**

(Manschadi et al. 2014)

- **Critical-P requirement**:

  Soil fertility level corresponding to 90-95% of maximum crop yield

  Soil P at this level is used with maximum efficiency

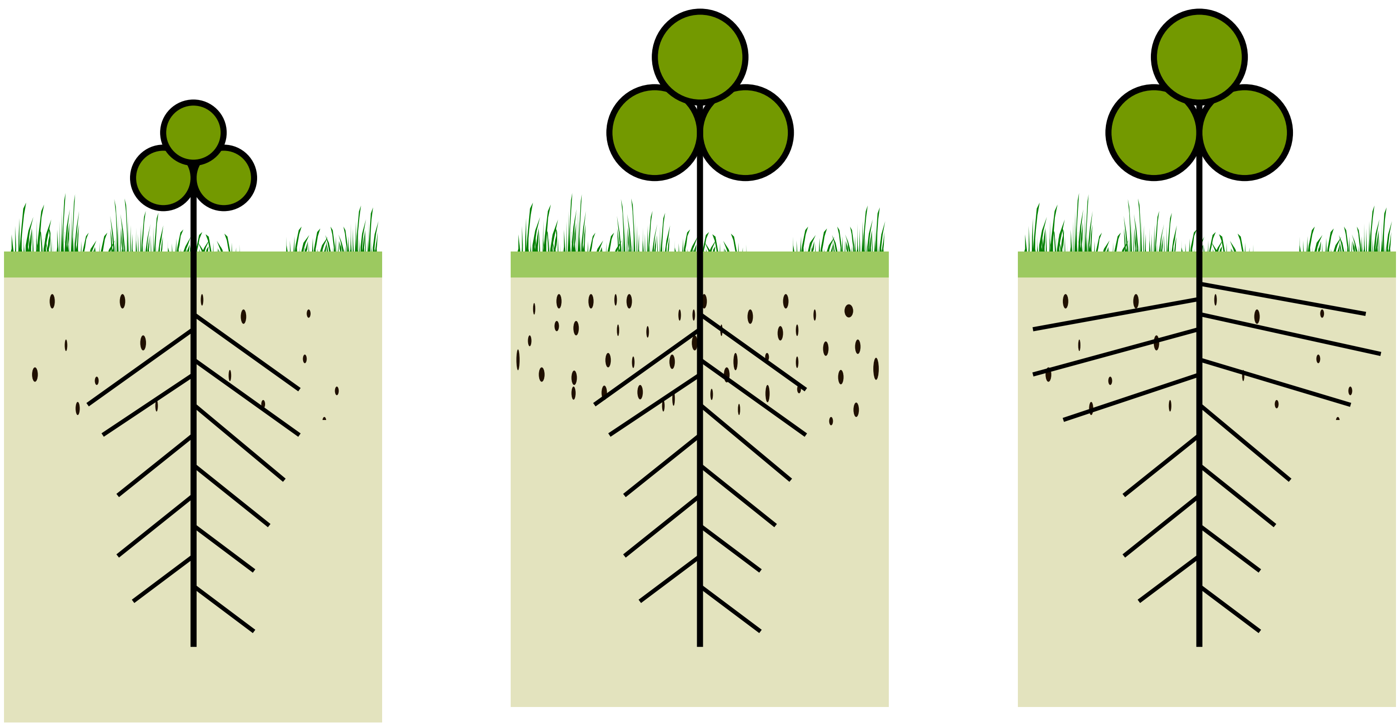
Standard genotype

P-efficient genotype

Soil P < Critical level

Soil P = Critical level

Lower external critical-P requirement



- P is highly immobile in soil
- Two basic plant strategies to acquire P
    - I. Mobilisation of P from poorly available P pools in the rhizosphere
    - II. Soil exploration

I. Root exudates & P mobilisation

II. Root phenes (traits) affecting soil exploration by roots

1. **Topsoil foraging**
    - Shallower root growth angle
    - Basal root whorl number
    - Adventitious rooting
    - Lateral branching
2. **Reducing the metabolic cost of soil exploration**
3. **Root etiolation**
4. **Root hairs**

Crop phenology

Root system architectures of common bean differing in basal root gravitropism

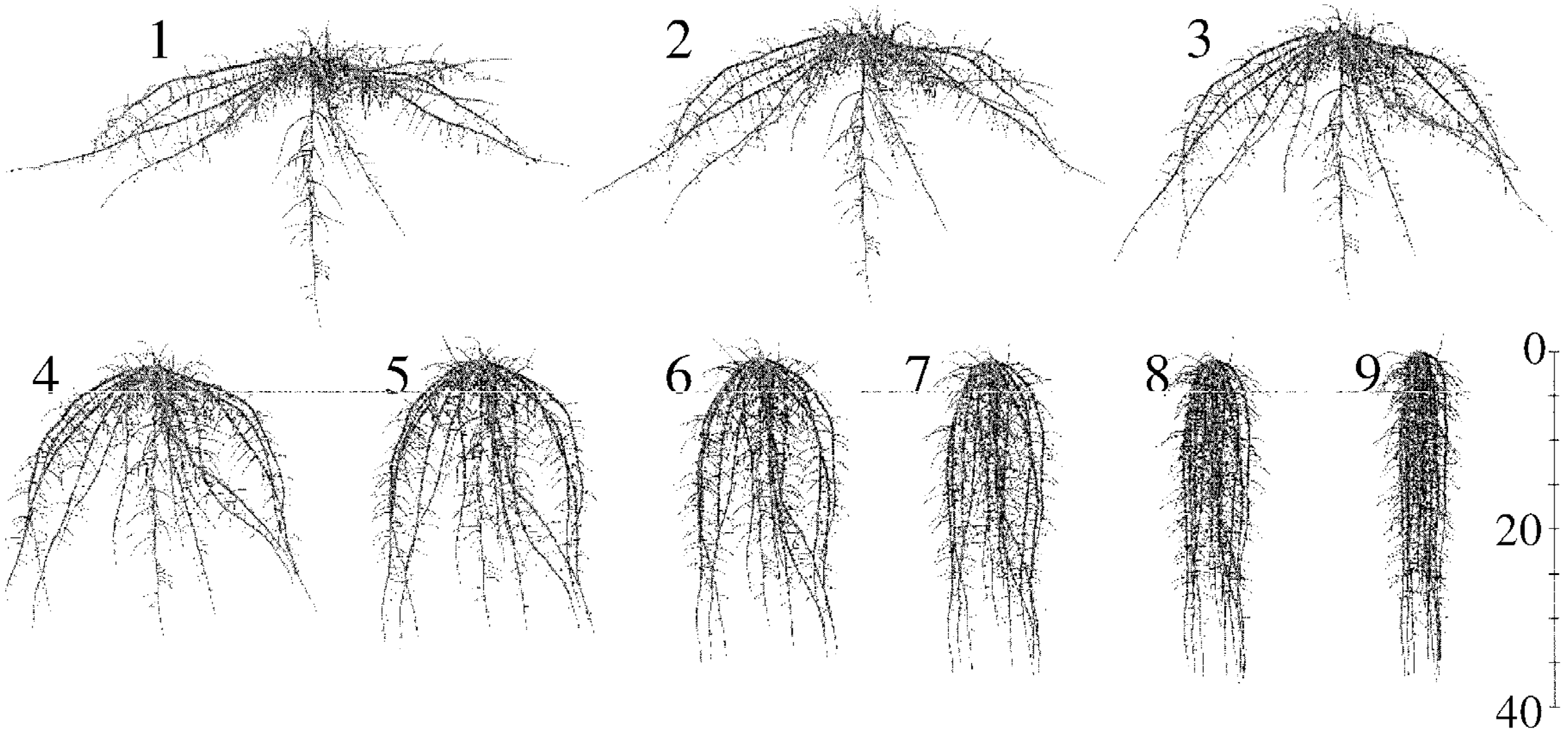
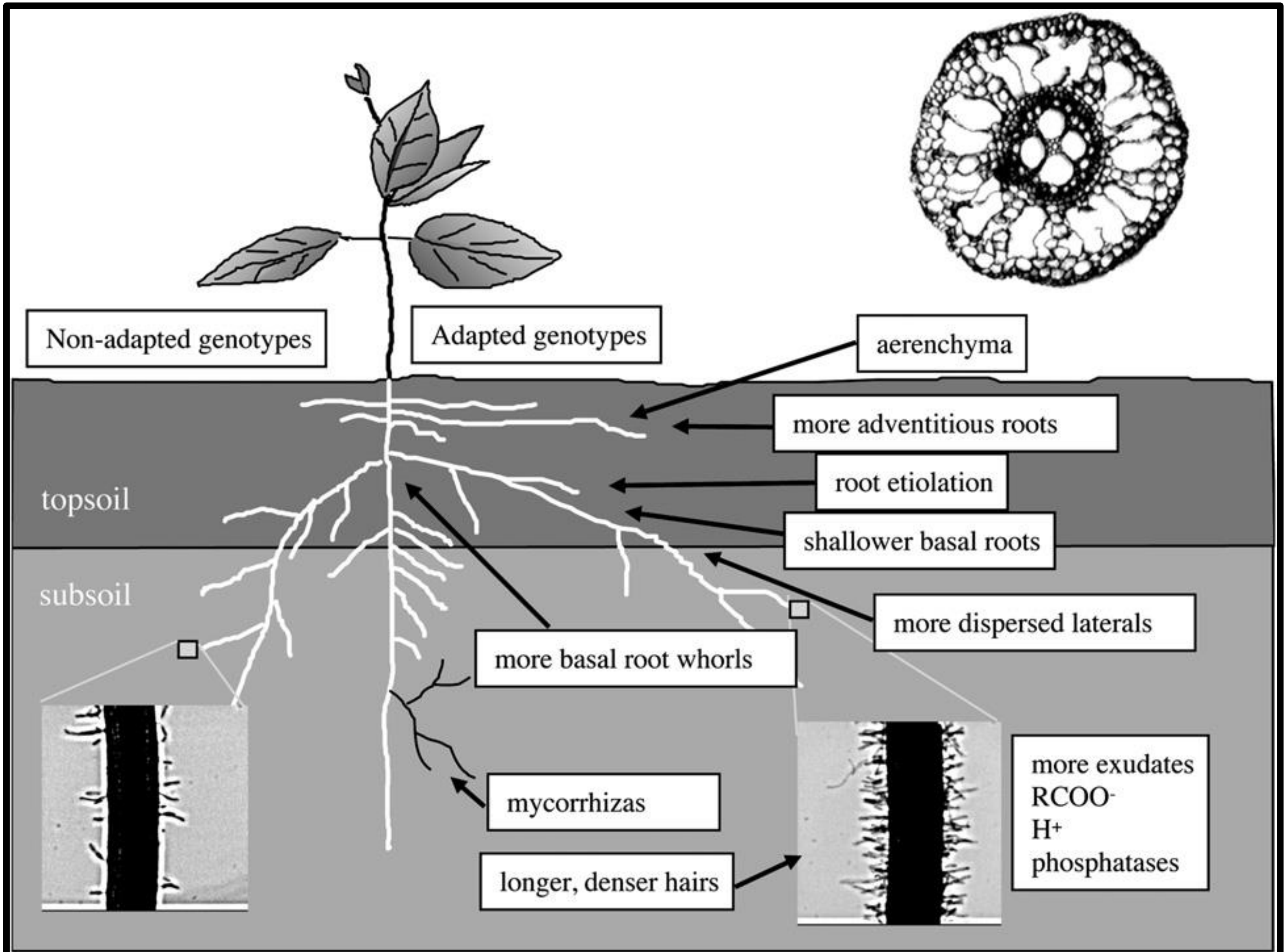


Figure 1. Simulated common bean root systems differing in the gravitropic growth of the basal roots, from shallow (number 1) to deep (number 9). These simulations are based on empirical measurements of the growth of the genotype Carioca (model number 5) at 320 hours after germination, then varied to simulate genotypic variation. The scale at the right shows depth from the seed placement in cm. This model shows the tap root and basal roots. Adventitious roots would develop above the basal roots from the hypocotyl (see Figure 6). Variation in the gravitropic growth of basal roots has a substantial influence on topsoil foraging by the root system. From Ge et al. (2000).

- In stratified soil: shallow RGA enhances topsoil foraging and P acquisition
- RGA accounts for up to 6-fold variation in P acquisition and 3-fold variation in bean yield in low-P soils
- **Shallow versus deep basal RGAs in two common bean genotypes grown in the field in South Africa**

(Lynch 2011)

- Root phenes associated with genotypic differences in adaptation to low P



(Lynch 2007)

- Screening soybean germplasm – 32 genotypes

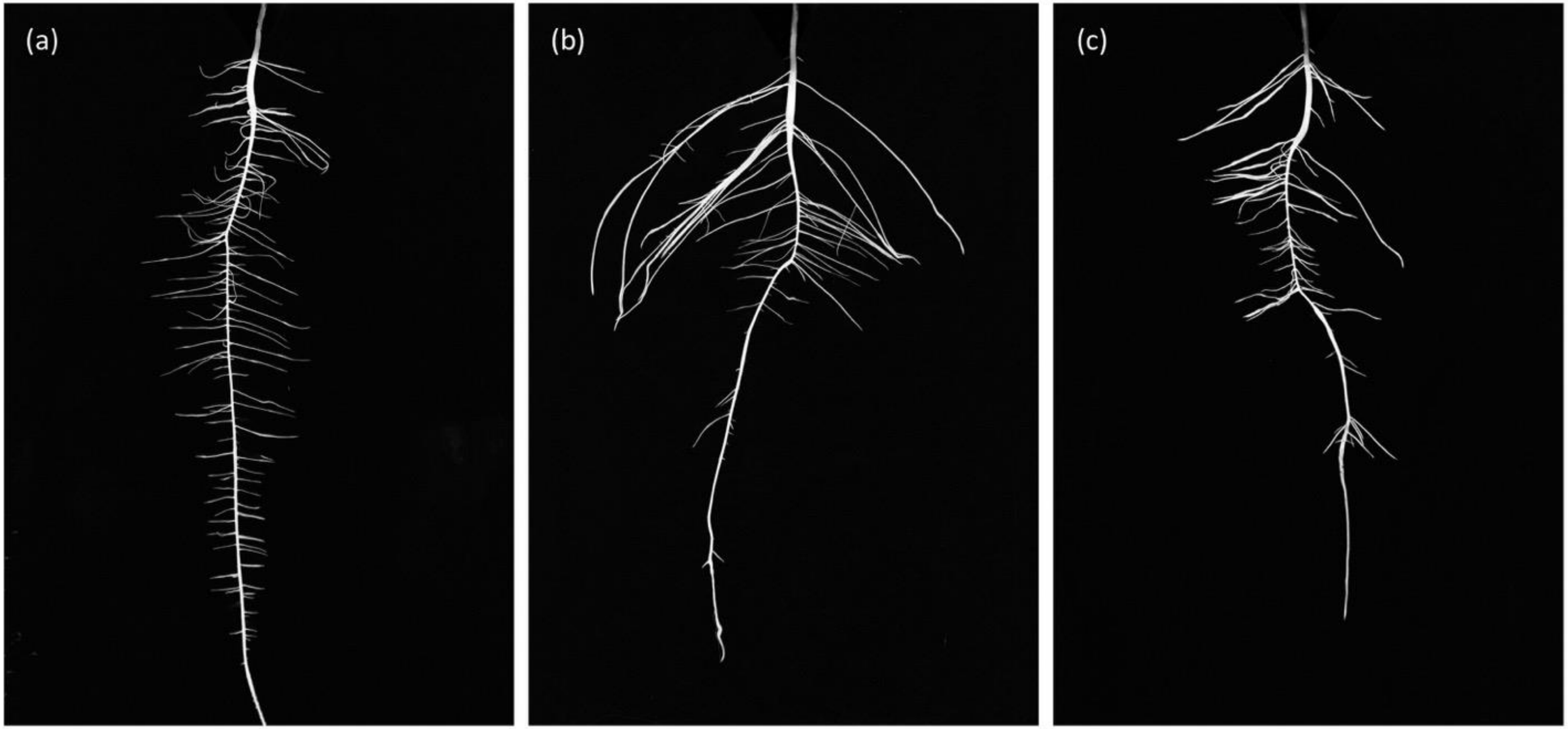
Table 1. Characteristics of soybean genotypes used in the screening for root traits.

Entry	Genotype	Seed source[a]	Maturity type[b]
1	Nanking	BJ 003	n.a.
2	Dornburger Stamm 106	SOJA 59/82	00
3	Altonagaarden	SOJA 345/81	0
4	Alurzynka	SOJA 587/80	n.a.
5	Fruhwirts Schwarze Pedoja	SOJA 53/88	n.a.
6	Zolta Przebedowska	SOJA 507/80	00
7	Gatersleben 31	SOJA 710/83	n.a.
8	Freitag Stamm 70	SOJA 24 A/82	000
9	Gatersleben 36	SOJA 451/83	n.a.
10	Heimkraft 1	SOJA 27/80	000
11	Brillmeyer Giesenska	SOJA 401/95	n.a.
12	Riede 525	SOJA 214/81	II
13	Amurskaja 41	SOJA 676/80	II
14	Ford	PI 548562	III
15	Chyazni No 2	SOJA 597/81	n.a.
16	Len-sin-pin-din	SOJA 702/80	0
17	Amurskaja Zlutozelená	SOJA 588/80	n.a.
18	Gatersleben 47	SOJA 698/80	n.a.
19	Gatersleben 48	SOJA 339/80	n.a.
20	Grignon 21	PI 438322	00
21	Bezencuskaja	SOJA 675/80	n.a.
22	Merit	PI 438364	0
23	Plaska Zlta Obravska	SOJA 494/88	0
24	Kyoto-Soy	JPN	0
25	ES Mentor	AUT	00
26	Christine	AUT	00
27	Suedina	AUT	00
28	Sigalia	AUT	00
29	GH8X-1	BOKU	00
30	GH8X-8	BOKU	00
31	GNN2X-111-15	BOKU	000
32	GNN2X-108-1	BOKU	000

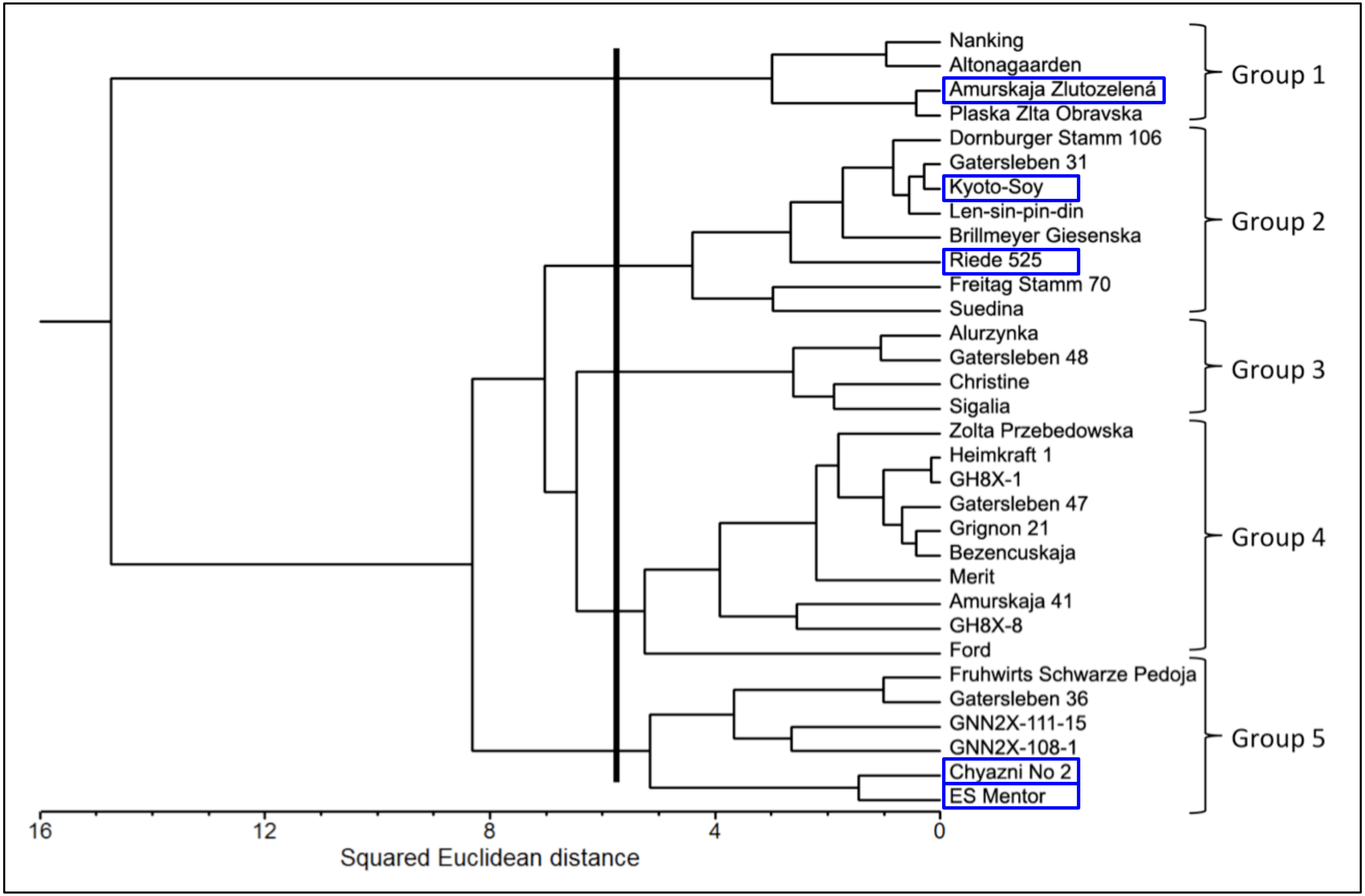
[a] BJ: Arche Noah gene bank (Schiltern, Austria)
SOJA: Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben, Germany)
PI: USDA Soybean Germplasm Collection (USDA-ARS, Beltsville, MD, USA)
JPN, AUT, and BOKU: Seeds received from Japan, Austria, and from the soybean breeding program at BOKU University, Vienna, Austria, respectively.
[b] n.a.: not assigned to a maturity group

- Root scans of 14-day old soybean genotypes

Riede 525 (a)
Amurskaja Zlutozelená (b)
ES Mentor (c)



- Clustering of soybean genotypes based on their BRGA, BRNO, RL, and RD



- Layered pot experiment

- Two different P-acquisition strategies
  - **Exploration (G4)**: vigorous and cost-effective root system (extensive network of thin roots)
  - **Exploitation (G5)**: enhanced P uptake per unit root length and dry mass (root hair development and rhizosphere modification processes)

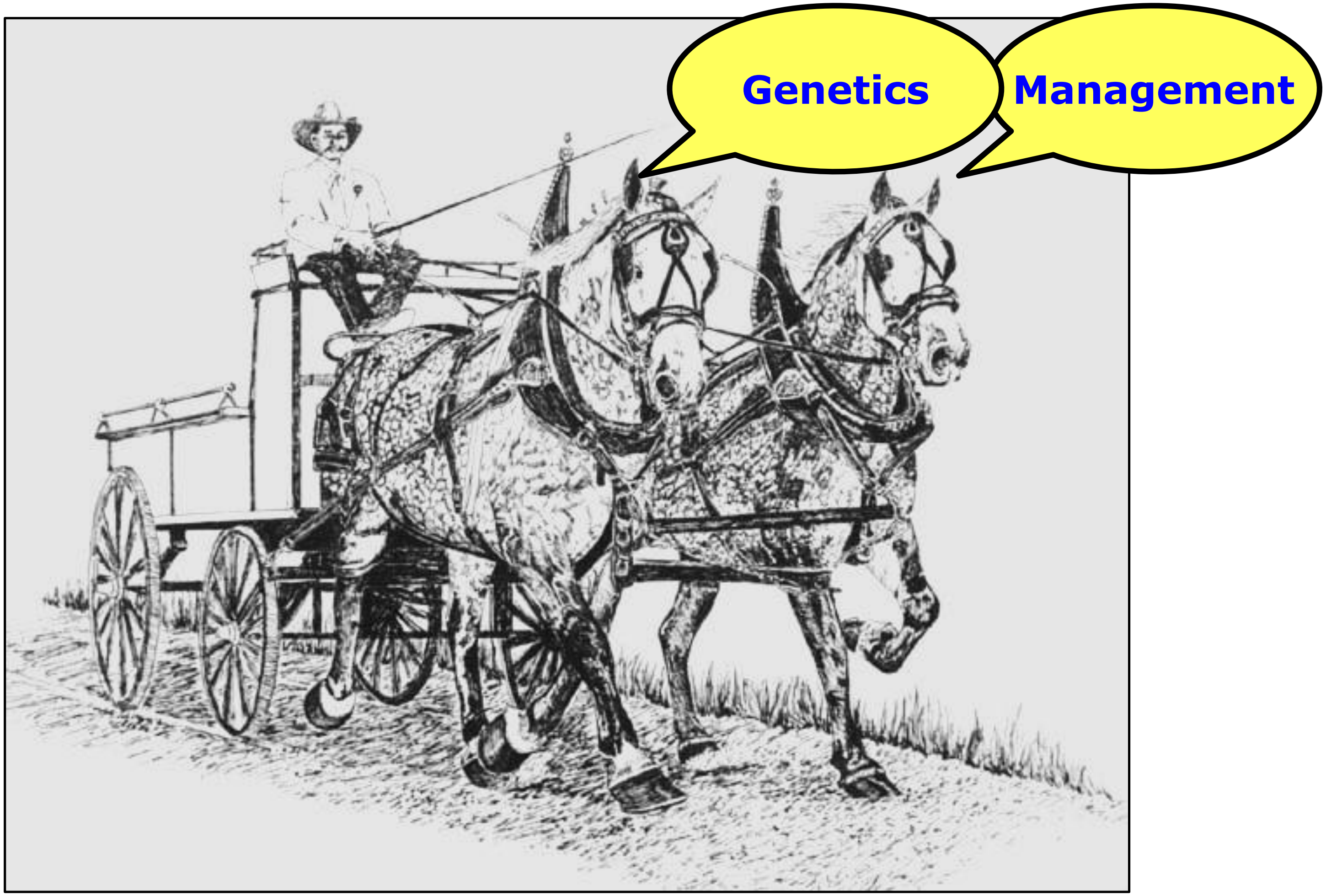
- Further research with RILs

- Utility of P-adaptive root phenes for crop yield under field conditions

- **Potential trade-offs and synergies for P acquisition, water uptake, symbiotic N fixation**
  - P in surface layers <-> N and water in subsoil
  - Root diameter: small diameter roots with greater SRL →
    increased volume of soil explored for water und nutrient;
    increased root hydraulic conductivity by decreasing the apoplastic
    barrier of water entering the xylem →
    superior ability for water uptake
    Ability for penetration of high-strength soil layers?

- Development & release of "**miracle wheat**" and "**miracle rice**"
- Improved plant genetics was the route to increased crop yields
- Research investment heavily favouring the **genetic approach**
- History of agricultural development: Green revolutions dependent on providing crops with greater resources (water, nutrients)
- Modern Green Revolution: "Chemical fertilizer is the fuel that has powered the Green Revolution's forward thrust" (**Borlaug, 1972**)

- **The "wagon" of yield increase is pulled by a pair of "horses"**
  - **Management**: increased availability of nitrogen (nutrients) and water
  - **Genetics**: plant genetic improvement to take advantage of greater resources



(Sinclair & Sinclair 2010)