Applied aspects of Molecular plant physiology

Christos Dordas
Professor
Head of the School of Agriculture
Aristotle University of Thessaloniki

Topics that will be covered

- Plant nutrition and specifically nitrogen
- Nitrogen use efficiency
- Ways of increasing N use efficiency
- Phenotyping
- Relationship between plant nutrition and human nutrition

Essential nutrients

- C, H, O
- Macronutrients:
  - **N, P, K**, Ca, Mg, S
- Micronutrients:
  - **Fe**, Cu, **Zn, Mn**, **B**, Cl, Mo, Ni
- Beneficial
  - Na, Si, Se, Co

Nitrogen

- 4º most abundant element in living organisms (plants 1.5-5 % DW).
- < 0,1% of the earth's crust
- 80 % of the atmosphere

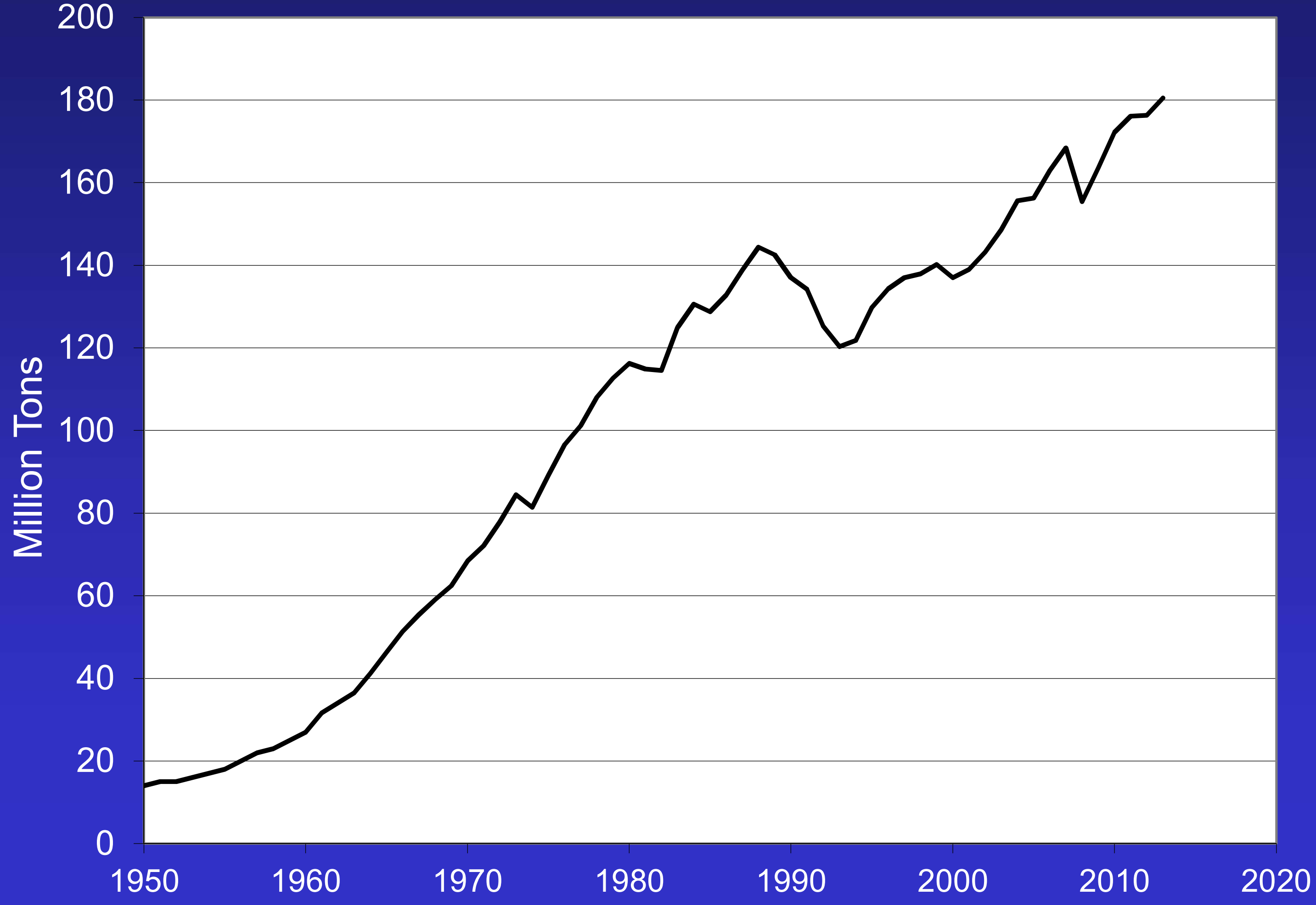
N deficiency

N deficiency

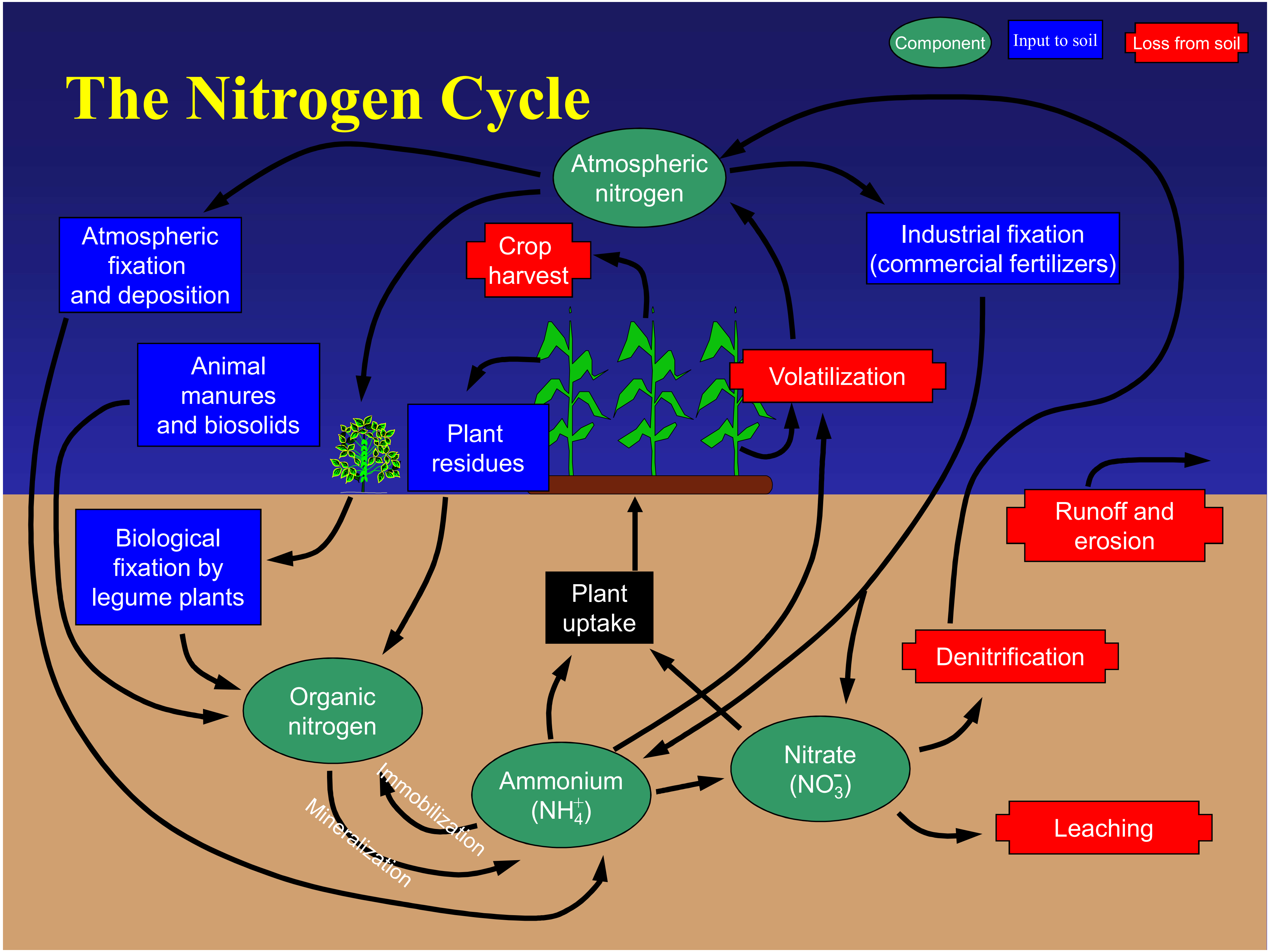
- N **+ N**

Nitrogen deficiency

Fertilizer consumption

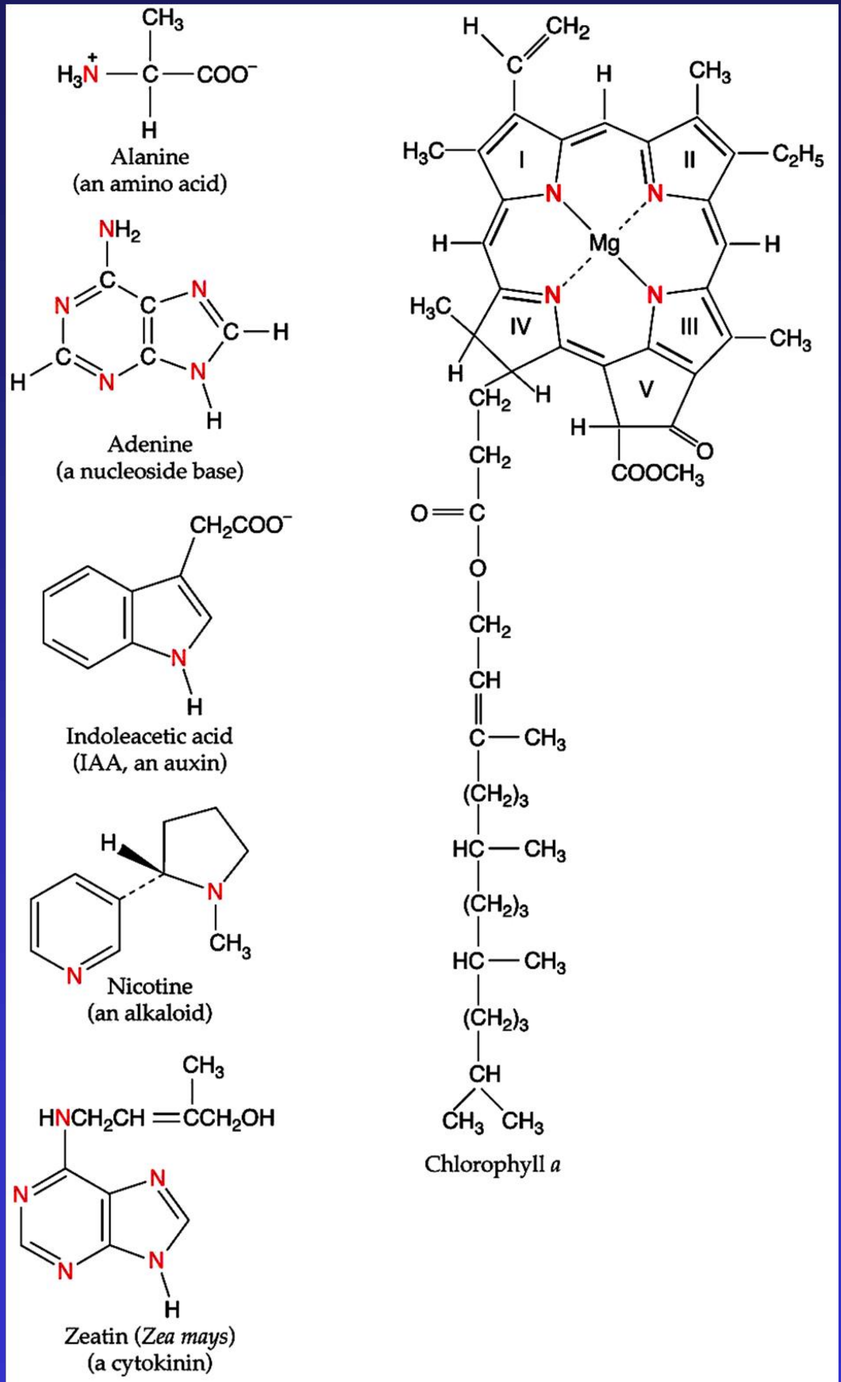


The Nitrogen Cycle



Inorganic N compounds

Compound	**Oxidation state of N**	**Name**
NH_3	-3	ammonia
NH_4	-3	ammonium ion
N_2	0	dinitrogen (nitrogen gas)
N_2O	+1	nitrous oxide
NO	+2	nitric oxide
NO_2^-	+3	nitrite
NO_2	+4	nitrogen dioxide
NO_3^-	+5	nitrate



Nitrogenous compounds

Nitrogen Uptake

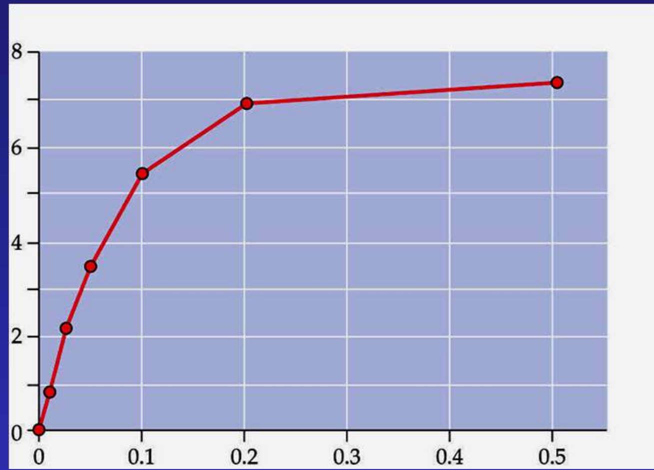
- NO_3^-
- NH_4^+
- Organic N (limited, not very important in agricultural ecosystems ???)
  - Urea
  - Amino acids (lysine, glycine and glutamate)
  - Oligopeptides
  - Purines
  - Nucleosides
  - Heterocyclic compounds (uric acid, xanthine, allantoin)

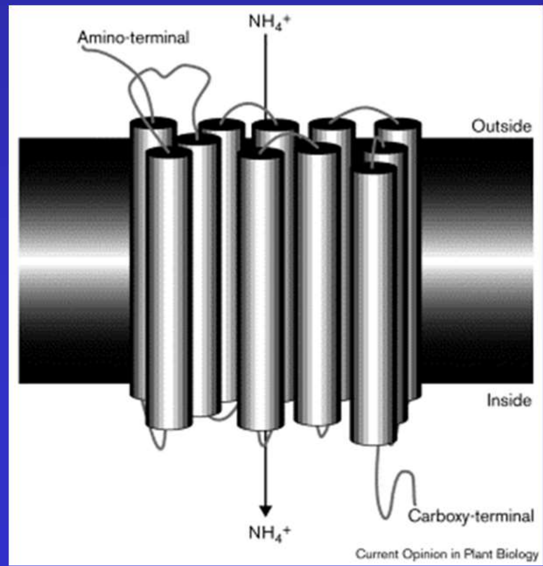
Factors affecting N uptake

- Internal
  - N and carbohydrate status
  - Plant species and stage of development
  - NH_4 induces inhibition of NO_3 uptake??
- External
  - Temperature
  - pH
  - Ion concentration in the external concentration
  - O_2

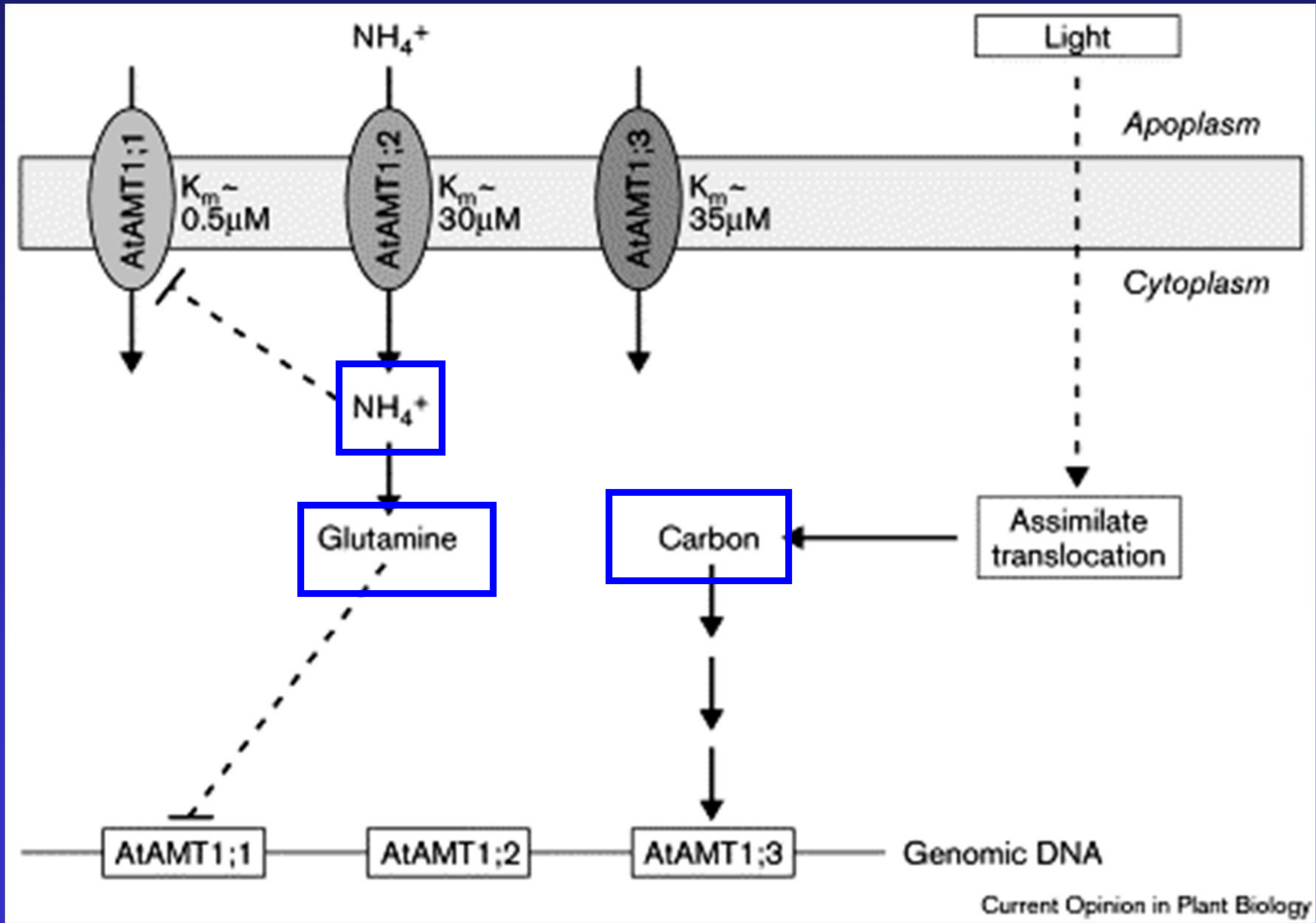
Ammonia uptake

- Acid soils
- Multiple transport system
- K_m 10-70 μM for NH_4^+
- Genes responsible for NH_4^+ uptake
  - AMT

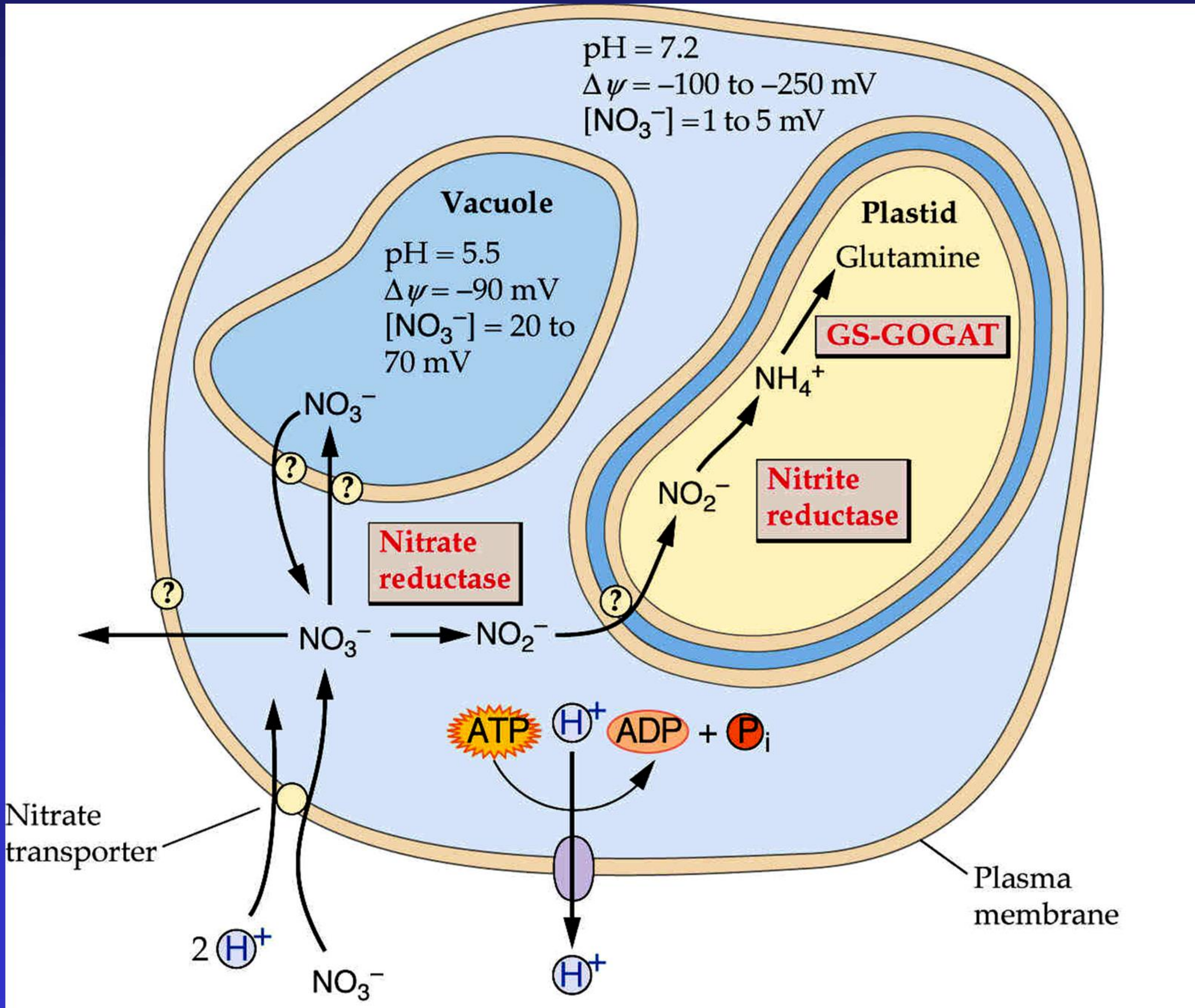




Regulation of AMT



Nitrate uptake



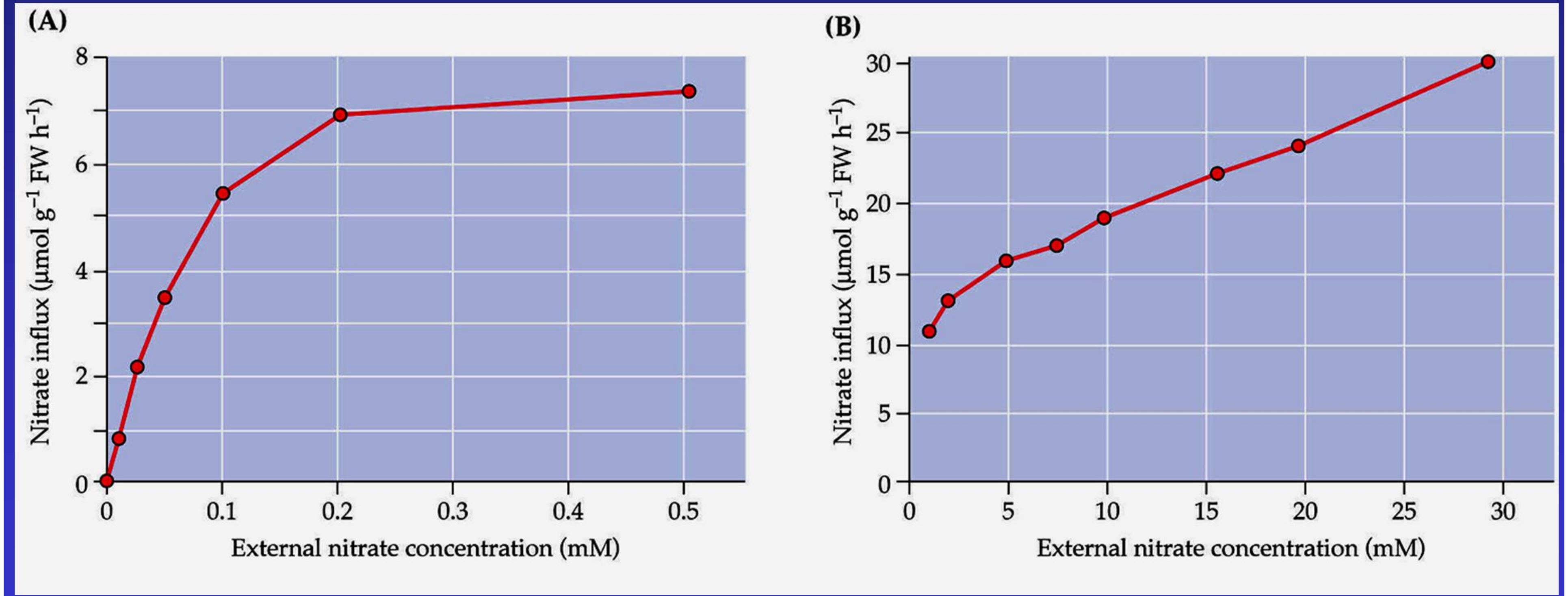
Nitrate uptake

- Main source of N
- Plants devote carbon and energy (ATP)
- NO_3
  - nutrient and signal,
  - affects plant metabolism and growth
- Major storage organs roots, stems, leaf midribs

Kinetics of nitrate uptake

High affinity transport system

Low affinity transport system



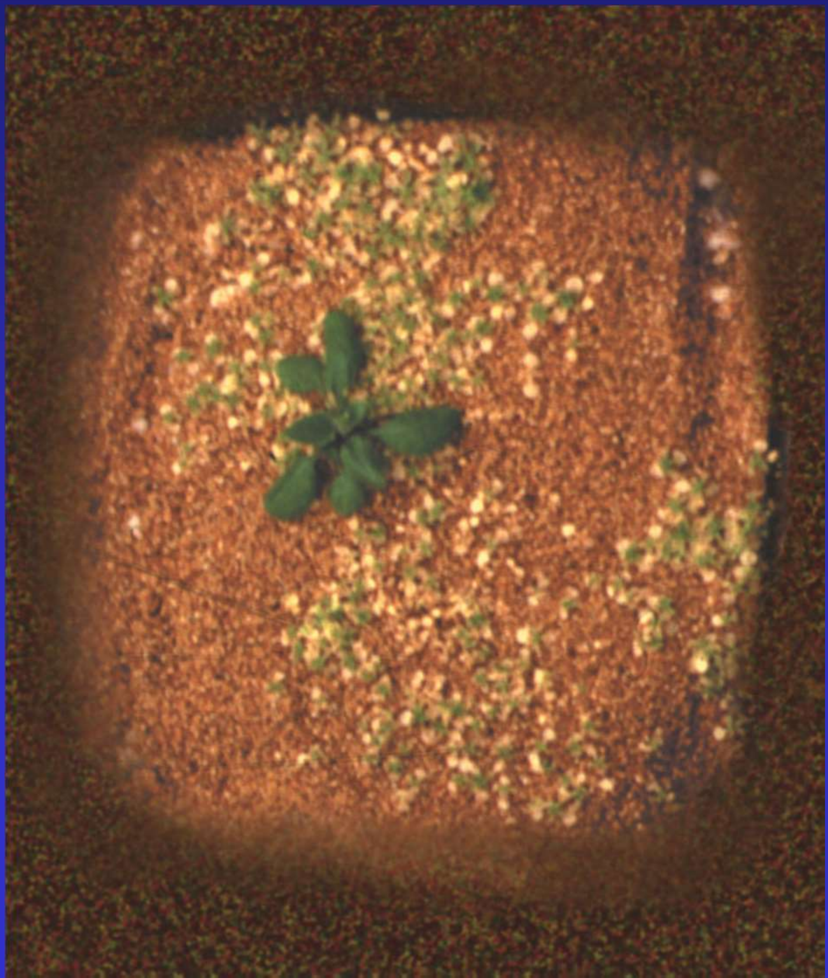
Nitrate uptake

- HATS
  - Mechanism I
  - Km 10-100 µM
  - Inducible
  - Constitutive component
- LATS
  - Mechanism II
  - >0,5 mM
  - Constitutive

Nitrate uptake

- Genes that are responsible for NO_3 uptake
  - NRT1
    - Dual affinity or low affinity
    - Expressed in outer layers of root (epidermal, cortical and endodermal cells)
  - NRT2
    - High affinity uptake system
    - Down regulation by reduced nitrogen (NH_4, glutamine)

Nitrate uptake mutants



Mutants are resistant to chlorate (ClO_3^-)

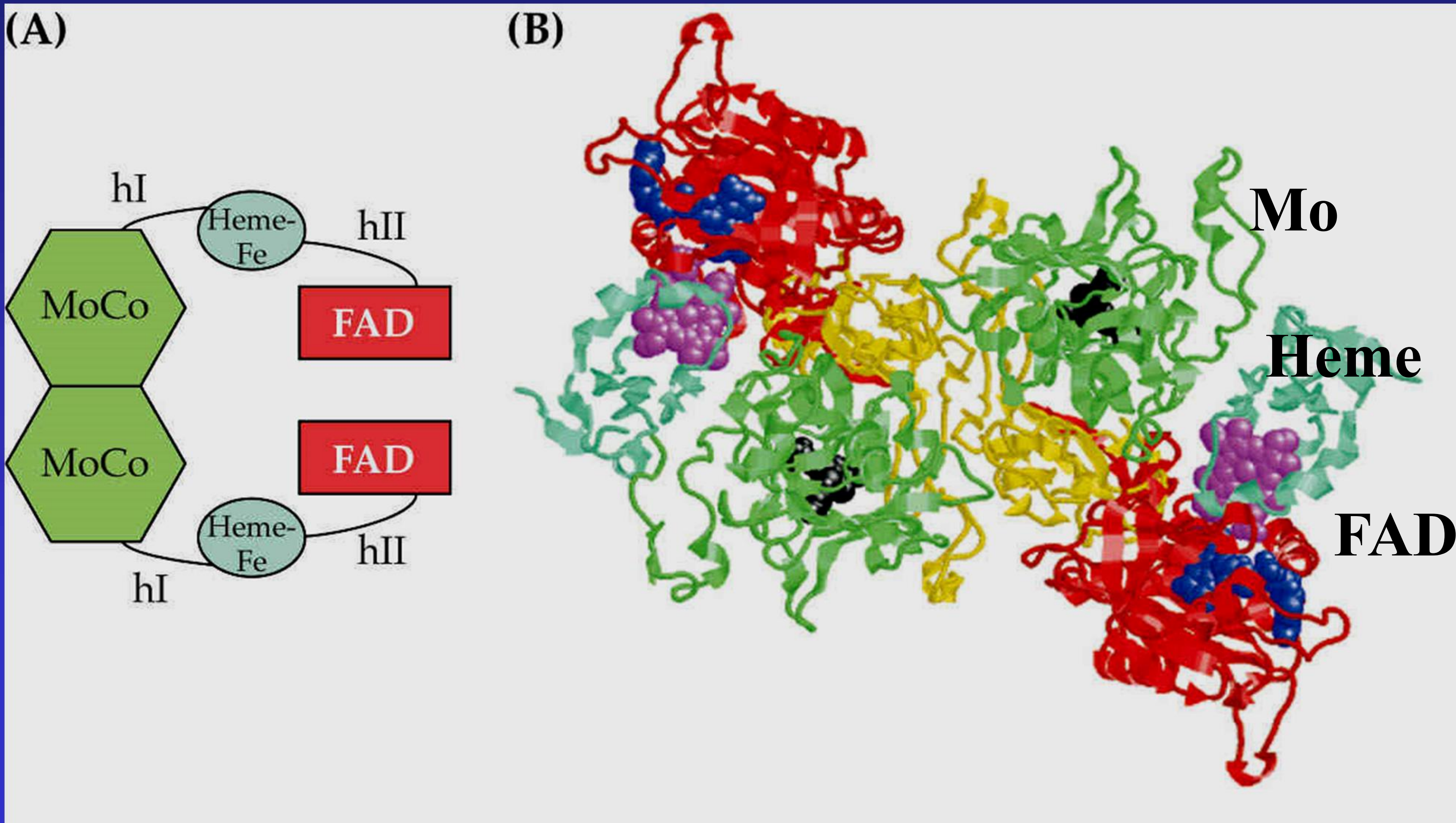
NO_3 reduction

- Species in which the root is the major site for reduction.
- Species exhibiting NO_3 reduction in both the root and the shoot.
- Species in which the shoot is the primary site for reduction.

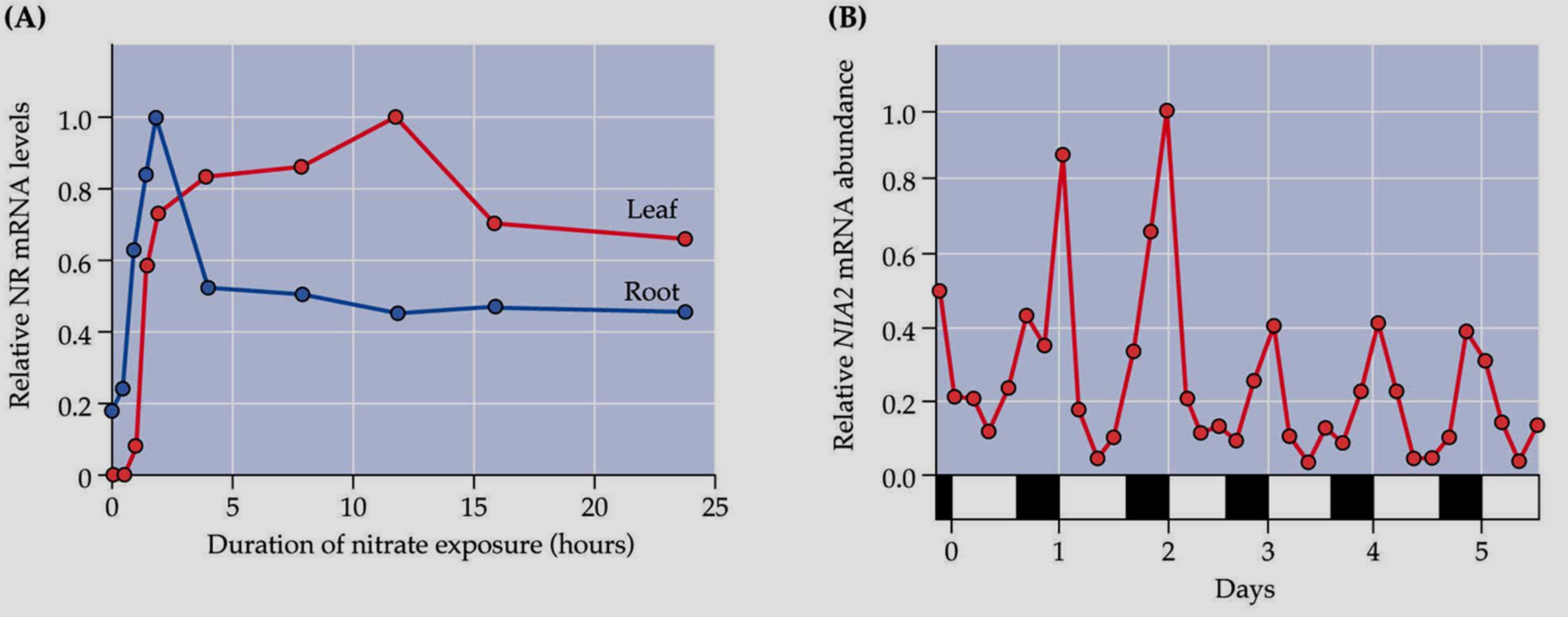
Nitrate reduction

- Nitrate reductase (NT)
- $NO_3^- + NAD(P)H + H^+ \longrightarrow NO_2^- + NAD(P) + H_2O$
  - Cofactors FAD, Heme-Fe, Mo
  - Each subunit is 1000 amino acids with three cofactors

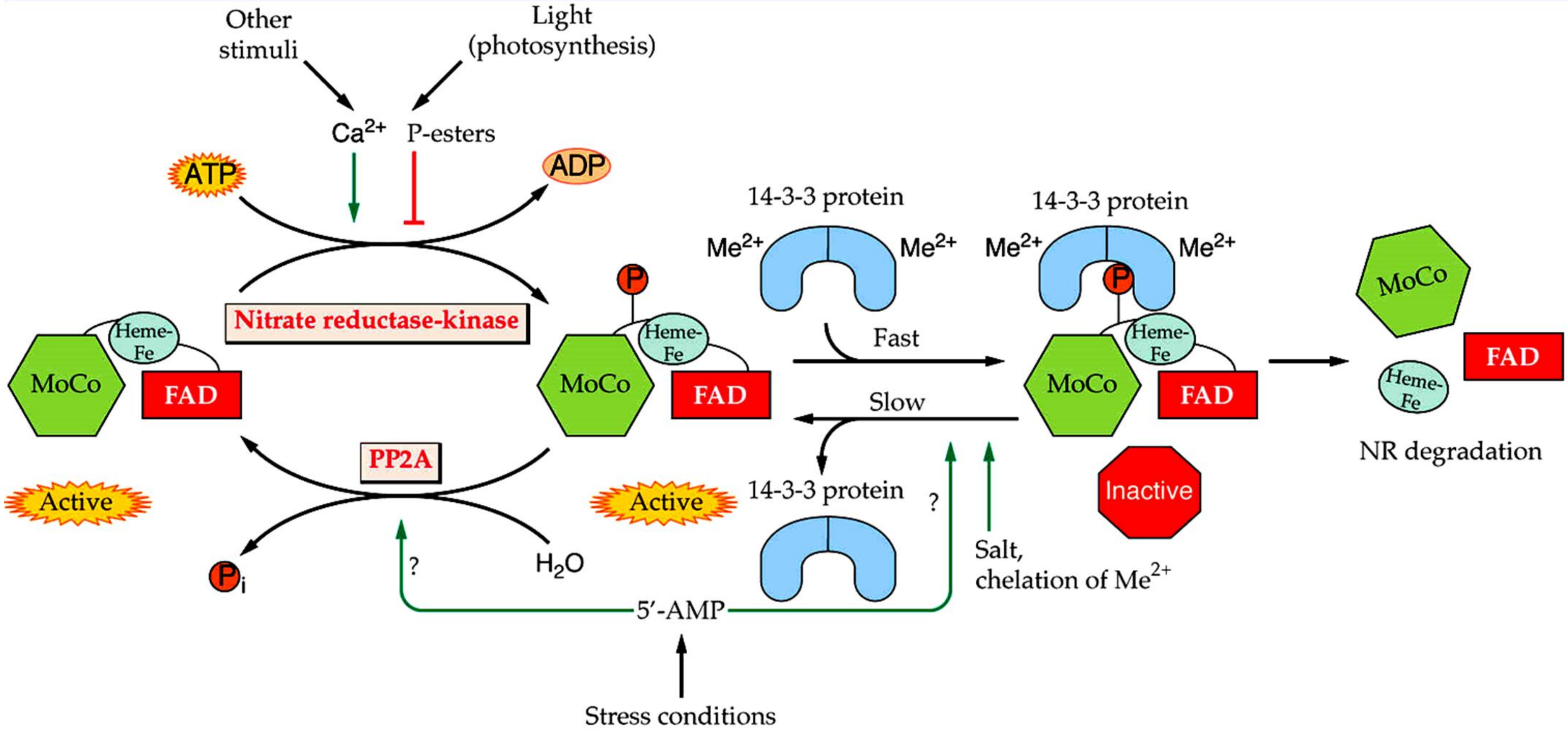
Nitrate reductase



Regulation of NR gene expression



Model for regulation of NR by phosphorylation



Signals that influence the NR expression

• Glutamine	Down regulates transcription
• Nitrogen starvation	Down regulates transcription
• Circadian rhythm	Modulates transcription depending on the time of the day
• Nitrate	Up-regulates transcription
• Cytokinin	Up-regulates transcription
• Sucrose	Up-regulates transcription
• Light	Up-regulates transcription
• Darkness	Down regulates transcription and activity
• High [CO_2]	Up-regulates activity
• Low [CO_2]	Down regulates activity
• Oxygen	Down regulates activity
• Anoxia	Up-regulates activity

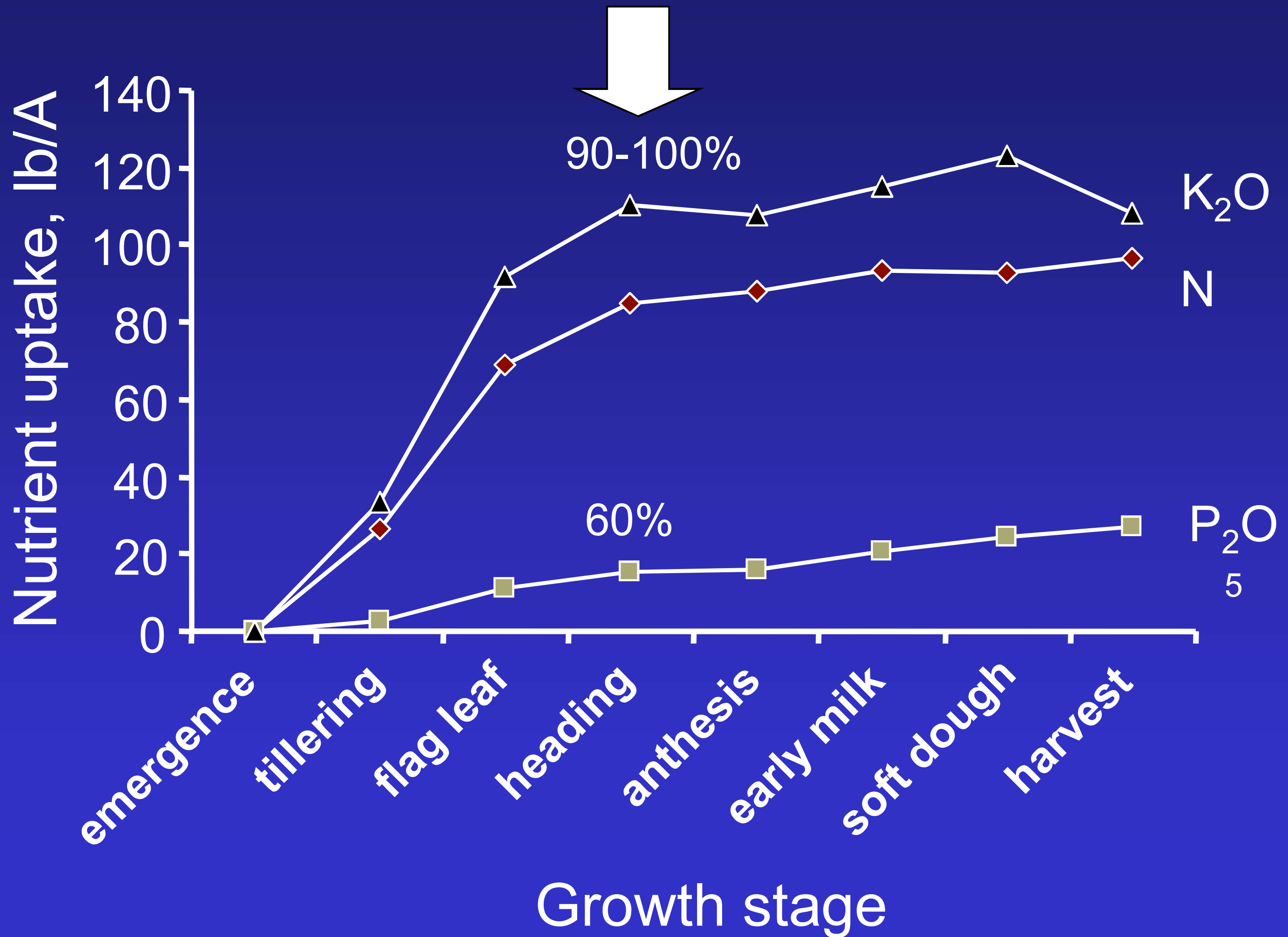
Whole plant physiology

- N accumulation and redistribution
- Roles of N for improving yield
- Nutrient use efficiency

Timing of N accumulation

- Factors
  - Daily fluctuation
  - Developmental stage
  - Species or cultivars
  - Availability of N
  - Planting date
  - Irrigation
  - Climate
  - stress

Pattern of N, P, and K uptake by wheat?



Johnston et al., 1999

N remobilization

- Grain contains 70 % of the total N in the plant at maturity
  - More than half is from remobilization from other plant parts.

Roles of N for improving yield

- Establishment of photosynthetic capacity
- Maintenance of photosynthetic capacity
- Establishment of sink capacity
- Maintenance of functional sinks

Nutrient Use Efficiency (NUE)

Nitrogen use efficiency (NUE)

- Definition of NUE
- Why the increased emphasis on NUE?
- **Sustainable** NUE

Nutrient use efficiency <u>functional</u> definitions

- NUE: grain yield per unit of applied N
- Physiological efficiency (PE): Dgrain yield/N uptake from fertilizer
  - $PE = (GY_{+N} - GY_{0N})/(UN_{+N} - UN_{0N})$
  - GY_{+N}= 4000 kg/ha, GY_{0N}=2800 kg/ha, difference in N uptake=60 kg/ha
  - PE=20 %
- Uptake efficiency (RE):DN uptake/DN fertilizer rate
  - $RE = (UN_{+N} - UN_{0N})/FN$
    - N uptake when no N applied = 30 kg/ha
    - N uptake when 100 kg applied = 90 kg/ha
    - RE=(90-30)/100 = 60%
- Agronomic efficiency (AE): Dgrain yield/N fertilizer rate
  - $AE = (GY_{+N} - GY_{0N})/FN$

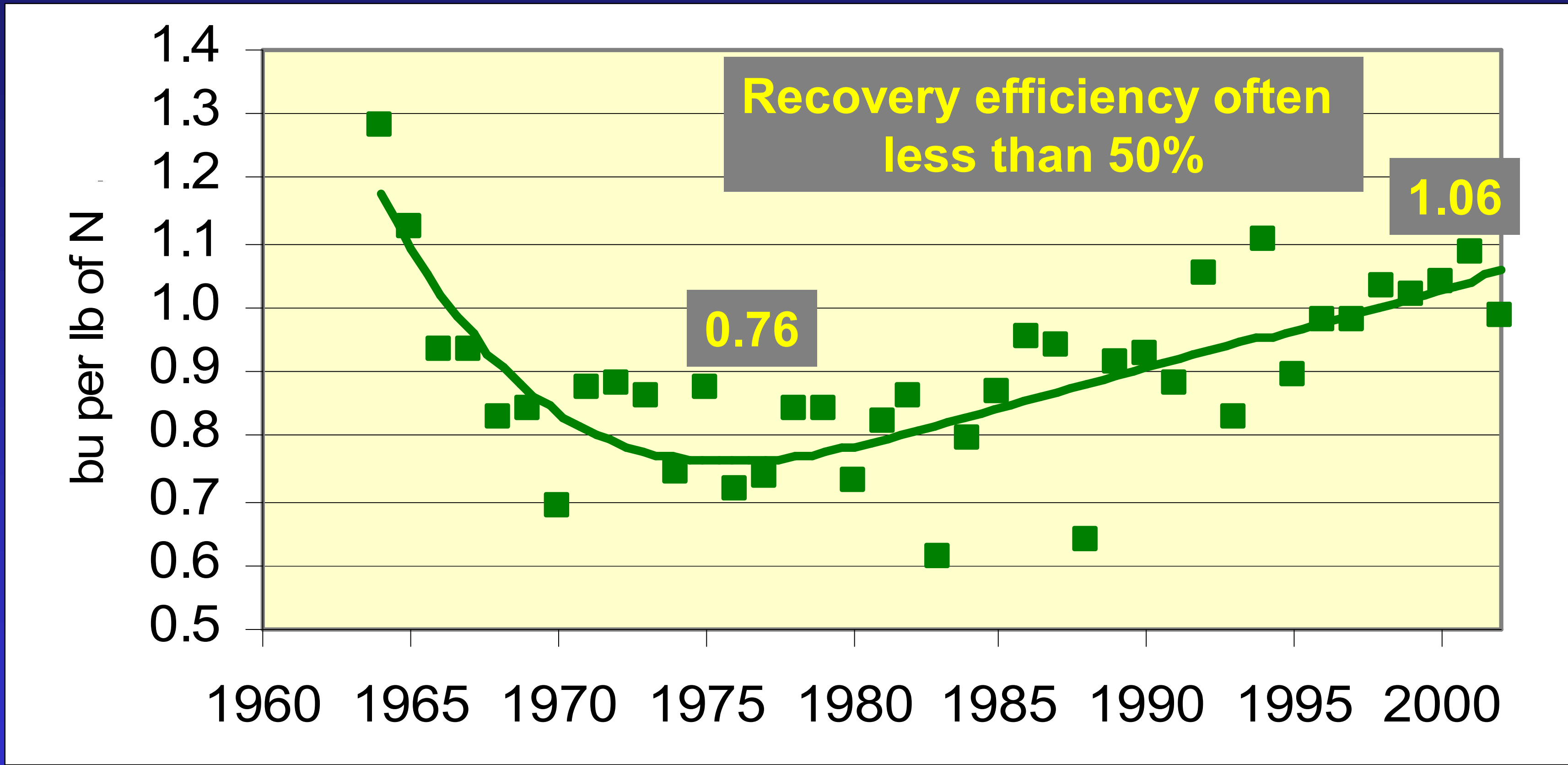
Nitrogen Use efficiency

- N availability
- Yield
- N recovered

Why the emphasis on NUE?

- **Input costs** are increasing
  - natural gas prices will remain higher than traditional levels … N prices will as well
- Increasing pressure to minimize negative **environmental impacts**
  - Global, national, state, and local levels
  - Water and air quality concerns
- **Government and EU incentive programs**
  - encouraging practices that increase NUE
- **Development and promotion of products** that promise increased NUE

Agronomic efficiency of fertilizer N used on corn grain in the U.S., 1964-2002



Since 1975:
39% increase in agronomic efficiency
12% increase in N fertilizer use
40% increase in corn yields

Factors affecting NUE

- Soil
  - Leaching, denitrification, volatilization and immobilization
  - N rate, N source, N placement and timing
- Plant
  - Absorption, translocation, assimilation, N redistribution
  - Plant characteristics (tissue N concentration, size and number of reproductive sinks)
  - Genotype

Genotypic differences in nutrient efficiency

Nutrient efficiency

a. Demand on cellular level (compartmentation, binding form)
b. Utilization within the shoot (eg retranslocation)
c. Seed reserves

a. Root-shoot transport (long distance transport)
b. Transport within the root (short distance transport)
c. Compartmentation/binding form within the root

Acquisition of nutrients

Acquisition of nutrients

Root morphology

Roots

- Inherent
- Response to deficiency

Mycorrhizas

Root physiology and biochemistry

a. Affinity of the uptake system (K_m)
b. Threshold concentration (C_{min})
c. Modification of the rhizosphere

Passive

Active

Response to deficiency

- eg cation-anion uptake ratio
- eg secretion of chelating, reducing compounds

Is maximum NUE our goal?

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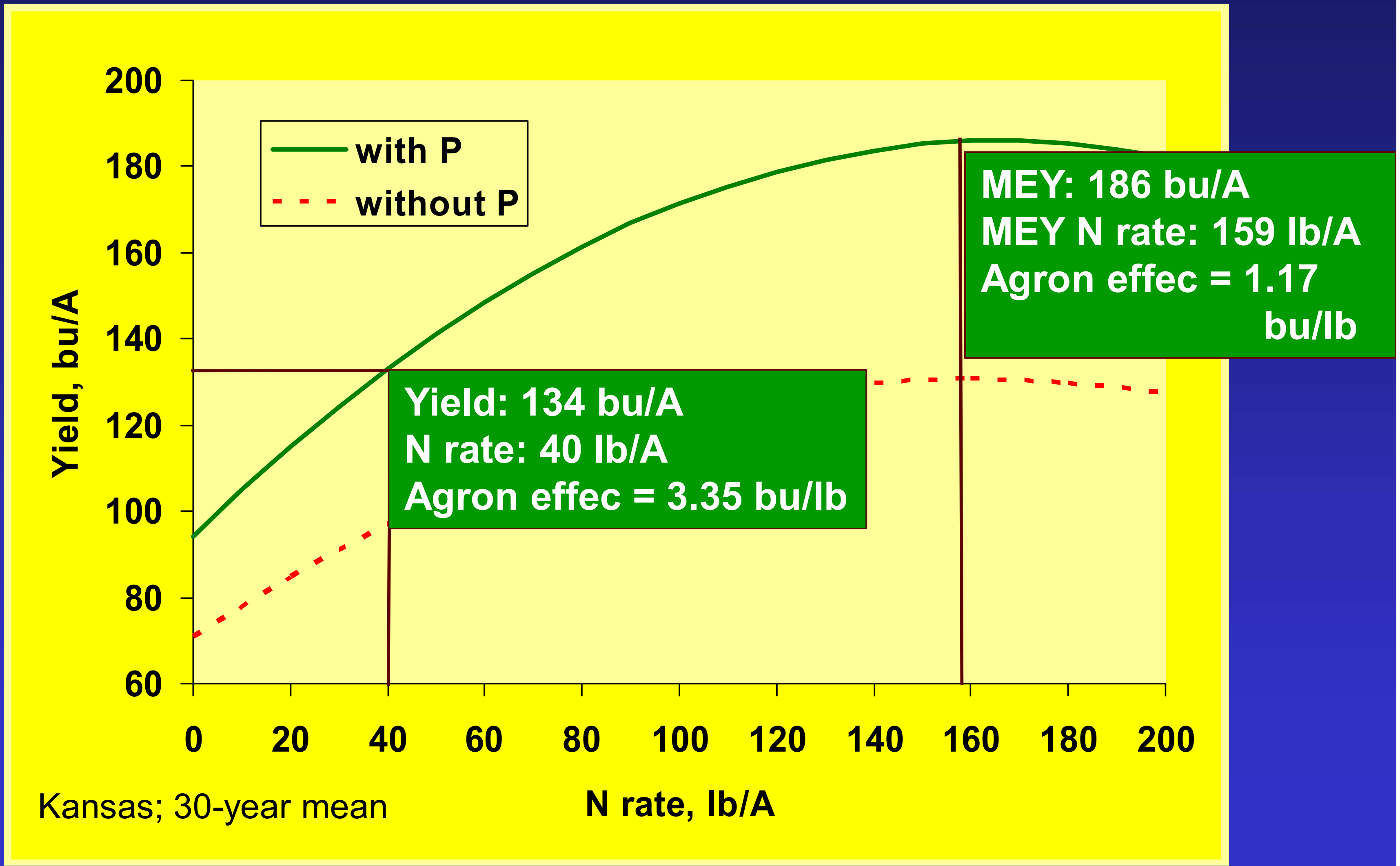


Source: Schlegel et al., 1996

Is maximum NUE our goal?

- **No (?)**
- Nutrient use should be **efficient and effective**
  - **Effective** – accomplishes the objectives of nutrient use
    - Meets production needs for **yield** and **quality**
    - Optimizes **profitability**
    - **Sustains** soil, water and air quality
      - Where **N** separates from **P and K**
- **Sustainable NUE** ……… our goal

Accurate estimates of N fertilizer efficiency are important!

- To **identify greatest opportunities** for increased efficiency by improved crop and soil management
- To identify **greatest N 'leaks' and leakage pathways** in the major cropping systems

Accurate estimates of N fertilizer efficiency are important!

- For improved estimates of global N balance and critical N loss thresholds for major cropping systems
  - Best available data from on-farm studies suggest that mean **N fertilizer uptake efficiency** is currently **less than 40%,** which is much lower **than estimates of 55%** used by others in constructing global N budgets, which would mean that **10 Mt N is unaccounted** for in global N budgets using inflated N uptake efficiency values
  - On-farm data on soil N balance and N losses are scarce

What is current status of N fertilizer efficiency?

- Estimates needed from **‘on-farm’ measurements**
  - Reliable data from the major cereal cropping systems
  - Measurements from **small research plots** tend overestimate current efficiencies by a large margin
- Available data indicate low N fertilizer uptake efficiency
  - Very low for rice
  - Low for maize despite improvements in recent years
  - Low to high on wheat depending on cropping system and yield levels

Nitrogen fertilizer uptake efficiency (RE) of maize, rice, and wheat based on on-farm measurements.

Crop (*rotation*)	Region	Number	N fertilizer ($kg\ ha^{-1}$) mean (+/- SD)	RE (% of applied) mean (+/- SD)
Maize (*maize-soybean*)	**USA**	**55**	**103 (85)**	**37 (30)**
Rice (*rice-rice*)	**Asia***	**179****	**117 (39)**	**31 (18)**
		179***	**112 (28)**	**40 (18)**
Wheat (*rice-wheat*)	**India**	**23^{+}**	**145 (31)**	**18 (11)**
		21^{++}	**123 (30)**	**49 (10)**

*** Six Asian countries. ** Farmers' practices. *** Field-specific management.**

$^{+}$ Low-yield year (1997, 2.3 $Mg\ ha^{-1}$). $^{++}$ High-yield year (1998, 4.8 $Mg\ ha^{-1}$).

Nitrogen Use Efficiency

- For cereal production is 33 %
- The rest 67 % represents a $ 16 billion annual loss of N fertilizer
  - Gaseous plant emission
  - soil denitrification (9,5 – 22 %)
  - surface runoff (1-13 %)
  - Volatilization (up to 40 %)
  - Leaching (20%)

How can NUE be increased?

- Rotations
- Forage Production systems
- Improved NUE due to cultivar (efficient genotypes)
- Conservation Tillage
- NH_4-N Source
- In season and foliar – Applied N
- Irrigation
- Precision Agriculture and Application resolution

Rotations

- Legumes have high NUE (cereal-legume)

Forage production systems

- Forage has lower plant gaseous N loss and higher NUE.
  - Do not approach flowering (where N losses are greater)
  - Wheat forage has NUE 77 % and grain only 33%
  - Corn forage NUE 70 %

Improved NUE due to cultivar

- Wheat cultivars have high harvest index, low plant loss and increased NUE
- High NUE has also been observed in rice varieties with high harvest index.
- Genetic selection under low N inputs in order to increase NUE

Conservation Tillage

- Erosion control, environment and operation costs and not in yield potential
- Subsurface placement of N fertilizer has the potential to significantly improve N availability and NUE

NH_4-N Source

- N uptake is higher 35 % (NH_4)
- Assimilation of N
  - NO_3 20 mol ATP/mol NO_3^-
  - NH_4 5 mol ATP/mol NH_4^+

In season and foliar – Applied N

- Preplant N reduces NUE
- Late season N increases grain protein and NUE
- Foliar applied N (at flowering) increases protein content and NUE

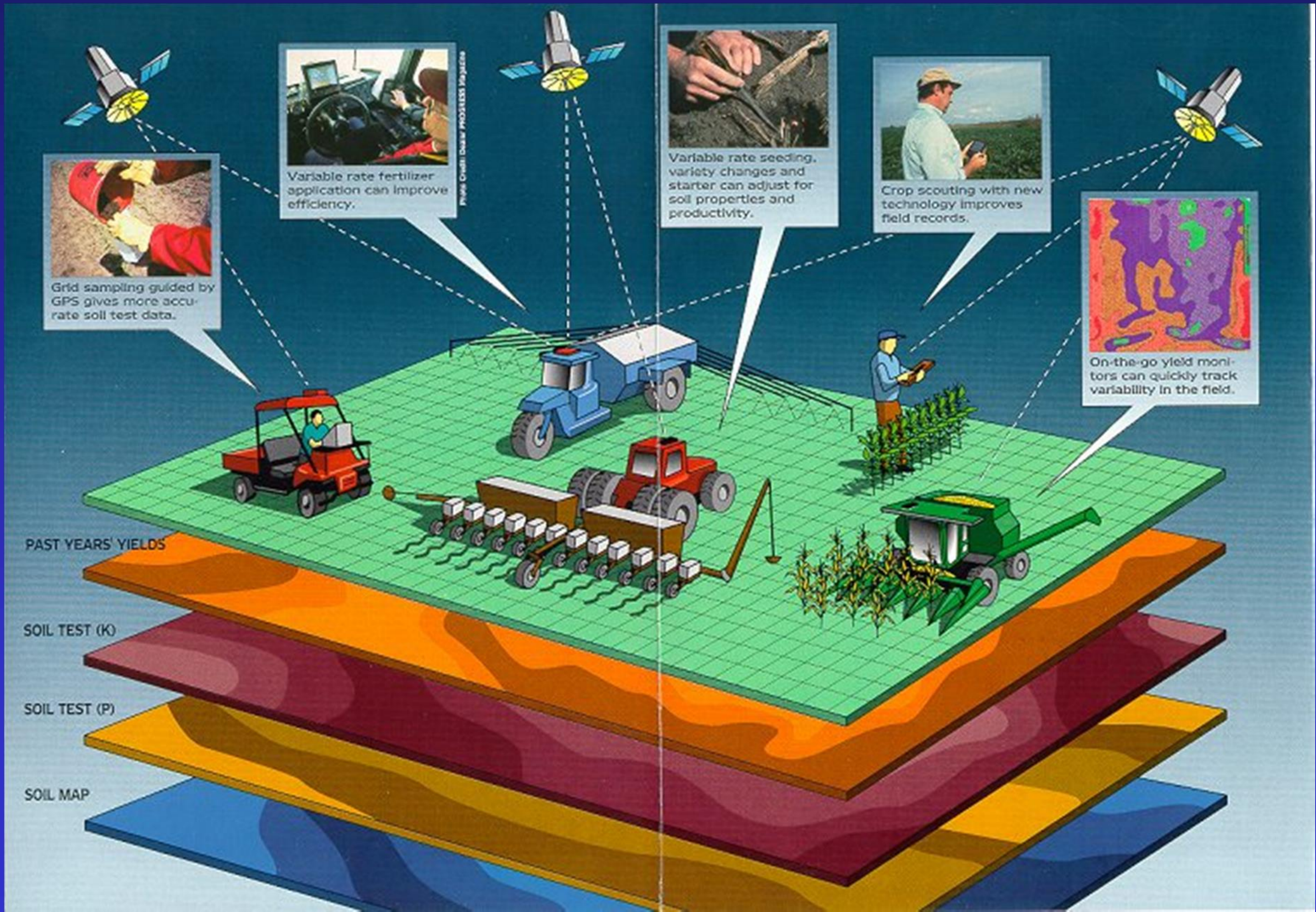
Irrigation

- Maximum NUE obtained with
  - Low N rates
  - Applied in season
  - With irrigation

Precision Agriculture and Application resolution

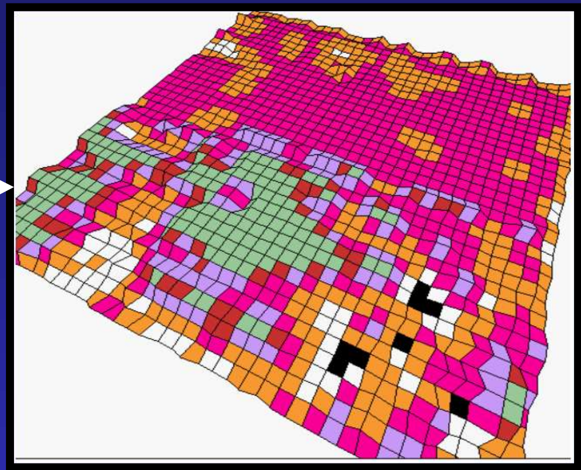
- **Timely** and **precise application** to meet plant needs
- Exact implementation of all **management operations** uniformly applied to a single field
- **Site-specific management** within a field to account for spatial variation in soil and pests
- **Crop management**: Variety/hybrid selection; tillage; planting date, density and row spacing; nutrient amount, formulation, and placement, integrated pest management; irrigation amount and timing

Site Specific Management: Accounting for spatial and temporal variability



Use of site-specific precision ag technologies

- Sensors
  - Remote (satellite)
  - Local (ground-based)

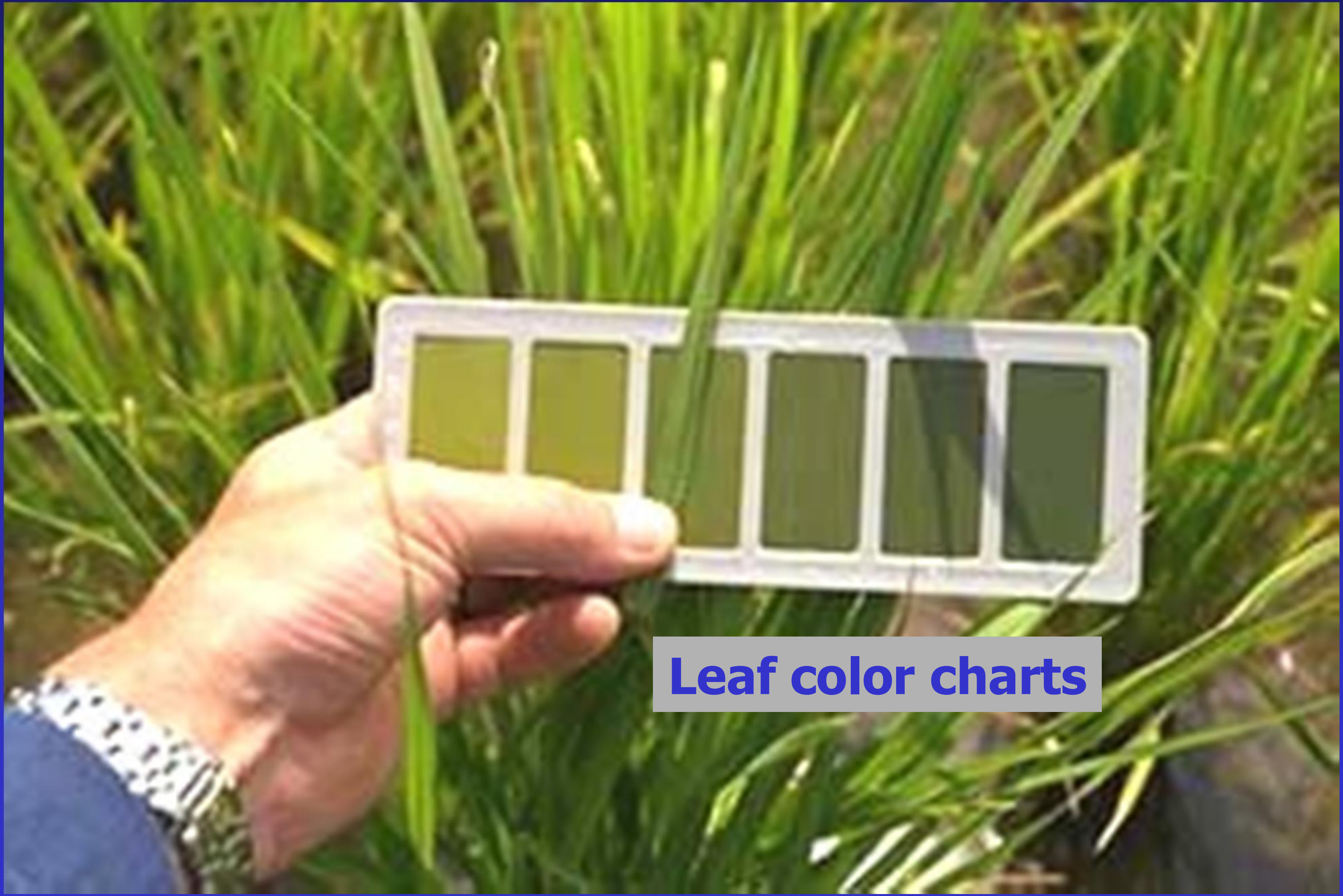


- Delayed application of a portion of planned N
  - Allows for variation in seasonal soil N mineralization
  - Integrated crop/weather models, soil tests

Example: GreenSeeker

NDVI Based

Less technology-intensive approach



Take Home Message

- High fertilizer N efficiency can be achieved at high yield levels and high rates of N fertilizer application only when applied N fertilizer is congruent with **crop N demand** and the **indigenous N supply**
  - Matches in-season pattern of crop N demand
  - Matches spatial variability within large production fields (mechanized agriculture)
  - Matches field-specific requirements in small production fields (labor-intensive agriculture)

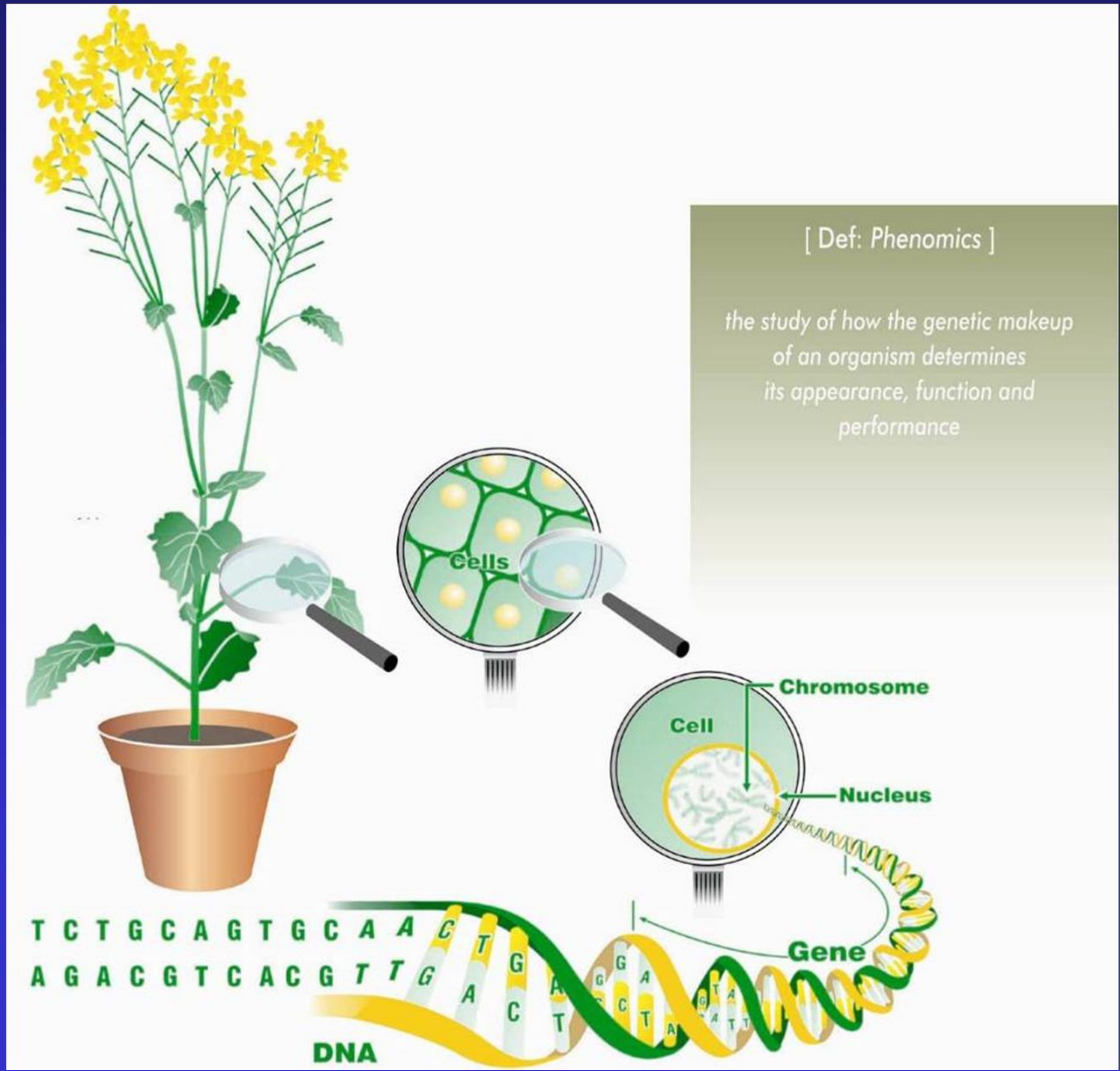
Conclusions

- Available technology can increase average N fertilizer uptake efficiency from **current 30-40% to 50%** while sustaining needed yield increases
  - Requires a large investment in adaptive research, extension, and perhaps farmer incentives to optimize profit and N efficiency
- Raising yields while ensuring protection of environmental quality may require efficiencies much **greater than 50%**
  - Requires new technologies and massive investment in both fundamental and applied research, extension, and farmer incentives

Conclusions (cont.)

- **Farmer incentives** are preferable to punitive regulations to avoid 'export' of cereal production to areas with least stringent environmental guidelines
- Critical need for data on **current levels of N fertilizer efficiency, N losses and loss pathways** from representative on-farm conditions in major cereal systems worldwide to help guide policy development and research investments
- Major expansion of crop production for **biofuels**

Plant phenomics



Some background information

A plant's **genotype** is all of its genes.

A plant's **phenotype** is how it looks and performs:

– a plant's phenotype is a combination of its genotype and the environment it grows in

– plants with the **same** genotype can have **different** phenotypes.

Phenotyping is analysing a plant's phenotype.

Phenomics is a way of speeding up phenotyping using high-tech imaging systems and computing power.

Why is plant phenomics important?

By **2050**, 9.1 billion people will populate the planet.

We will need to produce **70 per cent** more food to feed them, under tougher climate conditions.

This is one of humanity's greatest challenges.

How can we do it?

Two of the possible ways to help:

- Improve crop yields
- Breed crops that can cope with climate change

What does plant phenomics involve?

Phenomics borrows imaging techniques from medicine to allow researchers to study the inner workings of leaves, roots or whole plants.

Some phenomics techniques are:

- 3D imaging
- infrared and near-infrared imaging
- fluorescence imaging
- magnetic resonance imaging
- spectral reflectance.

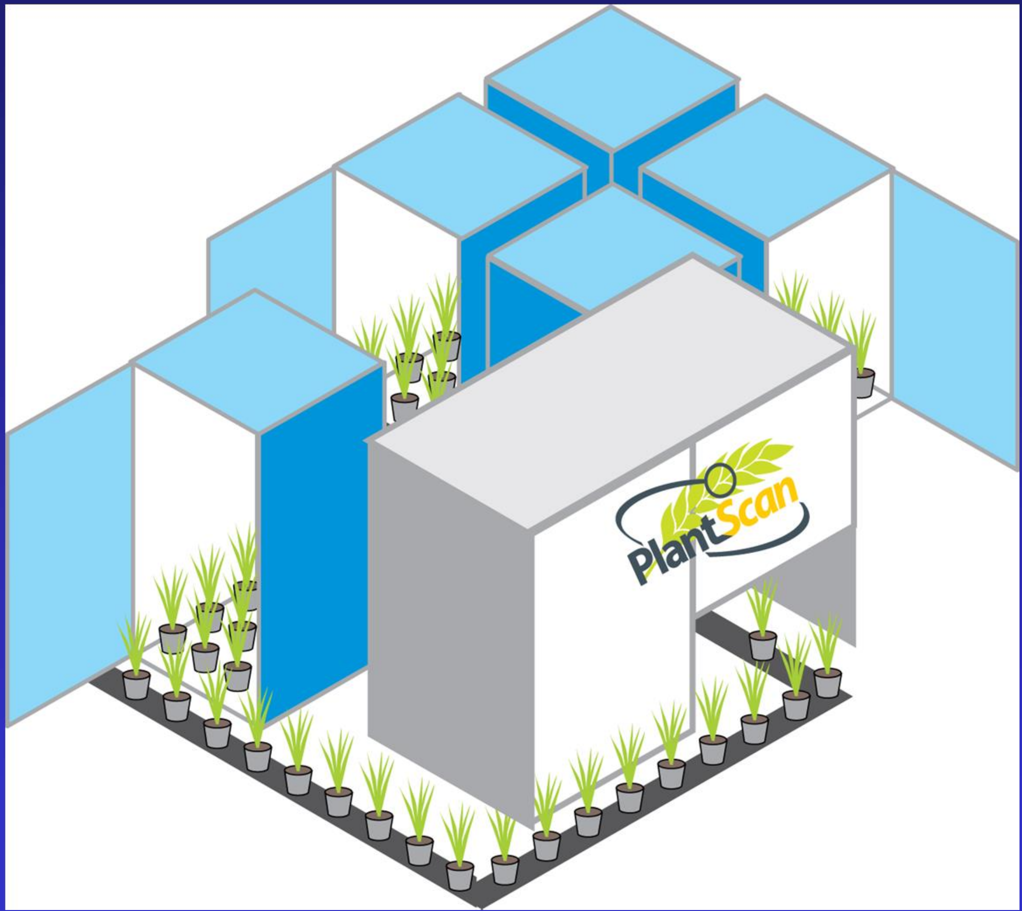
Three-dimensional (3D) imaging

Digital photos of the top and sides of plants are combined into a 3D image.

Measurements that can be taken using a 3D image include:

- shoot mass
- leaf number, shape and angle
- leaf colour
- leaf health.

Three-dimensional (3D) imaging

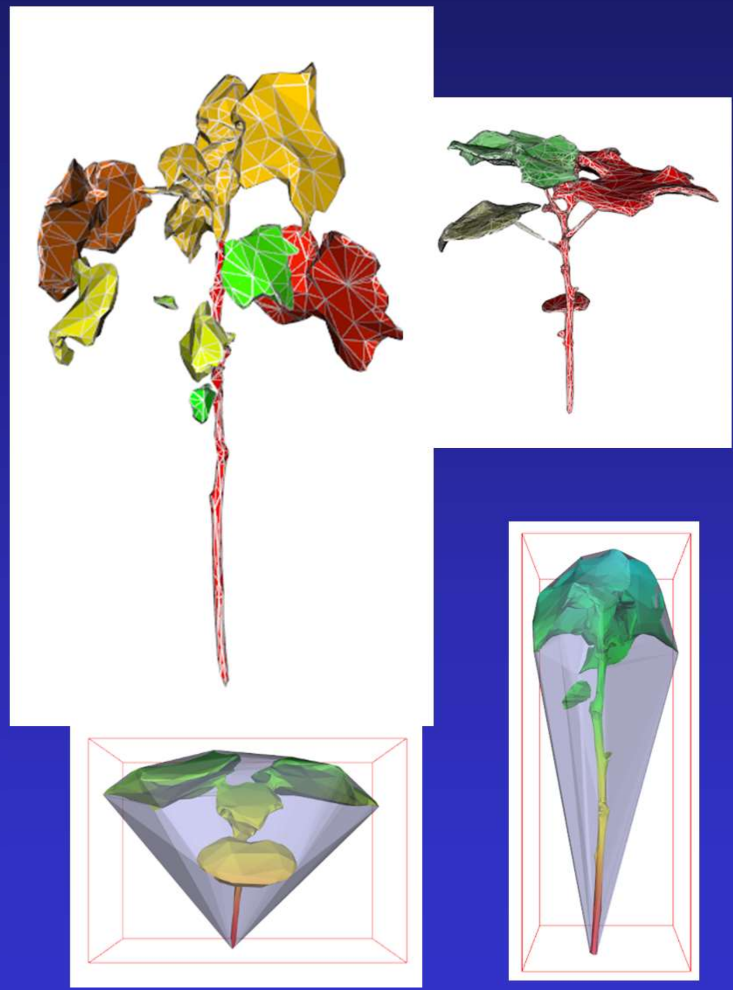


Pots of plants move on a conveyor belt through an imaging chamber.

The 3D models are automatically generated by a computer program.

Three-dimensional (3D) imaging

A cotton plant prepared for imaging (above), and 3D models (right)



Jurgen Fripp CSIRO ICT E-Health Brisbane

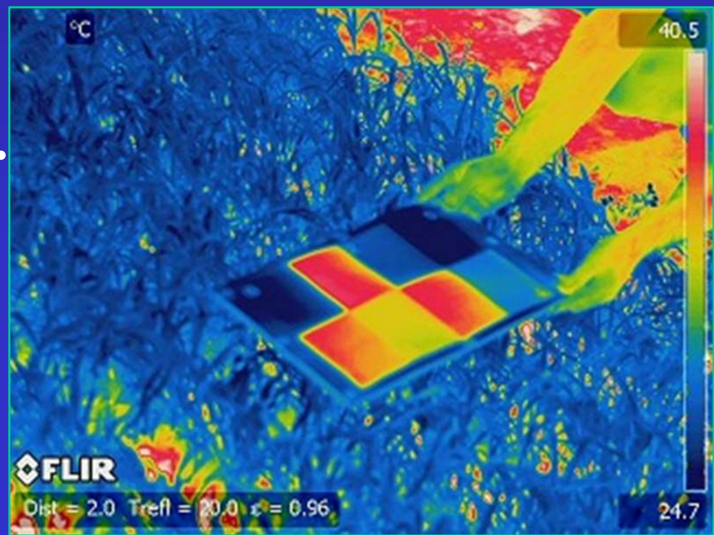
Far infrared (FIR) imaging

FIR cameras are used to study temperature.

- They use light in the FIR region of the spectrum (15–1000 µm).

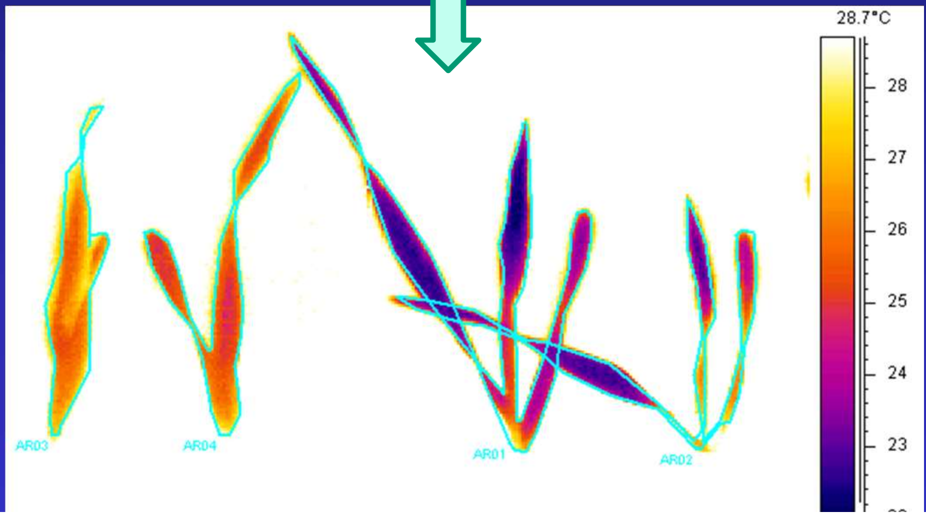
Temperature differences can be used to study:

- salinity tolerance
- water usage
- photosynthesis efficiency.



Far infrared (FIR) imaging

Cooler plants have better root systems and take up more water.

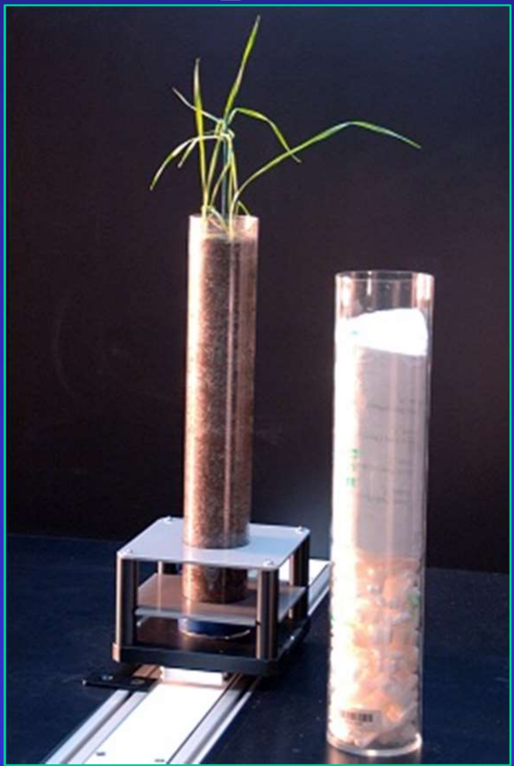


FIR imaging can be used for individual plants or for whole crops.

Near infrared (NIR) imaging

Near-infrared (NIR) cameras study water content and movement in leaves and soil.

- They use light in the NIR region of the spectrum (900–1550 μm)



Plants are grown in clear pots so roots can be photographed while the plant is growing.

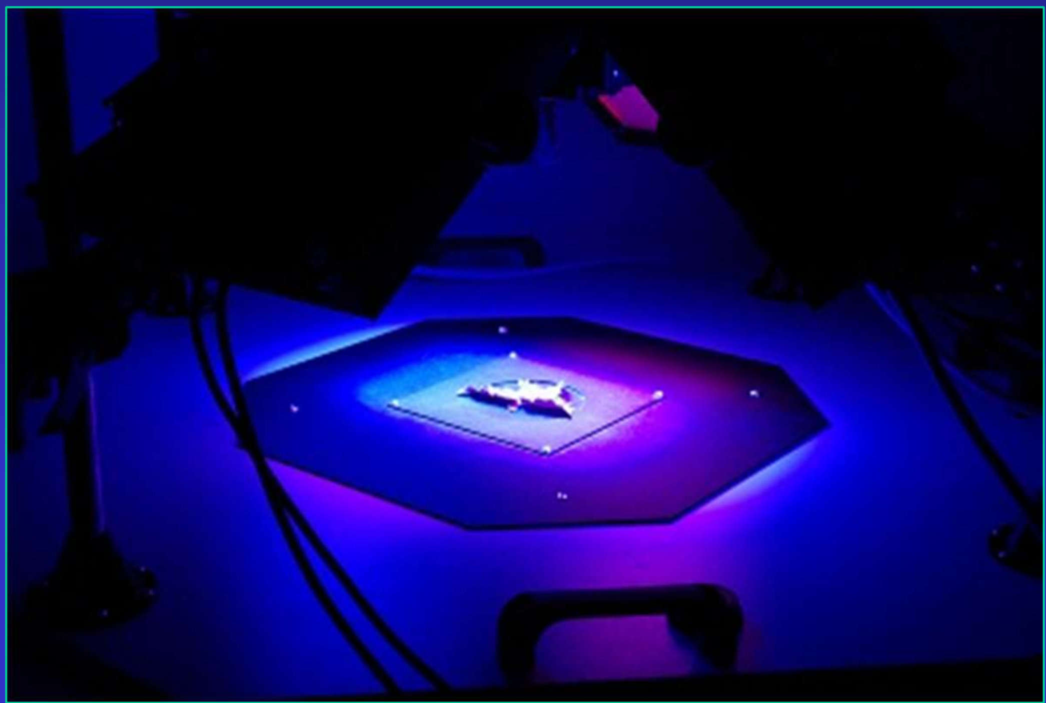
Soil NIR measurements are used to calculate:

- how much water the roots remove from the soil
- where and how much water the plant is using.

Fluorescence imaging

Fluorescence imaging is used to study plant health and photosynthesis.

- Fluorescence occurs when an object absorbs light of one wavelength and gives off light of a different wavelength.



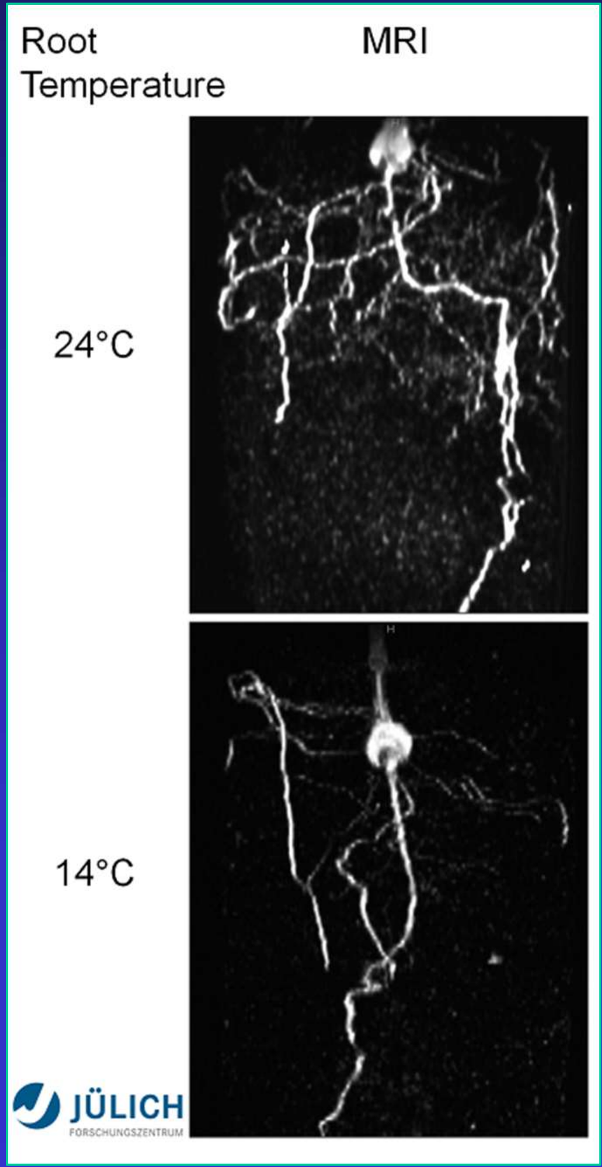
Chlorophyll fluorescence is used to study the effect of different genes or environmental conditions on the efficiency of photosynthesis.

Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) is used to study plant roots.

- MRI uses a magnetic field and radio waves to take images of roots in the same way as for imaging body organs in medicine.

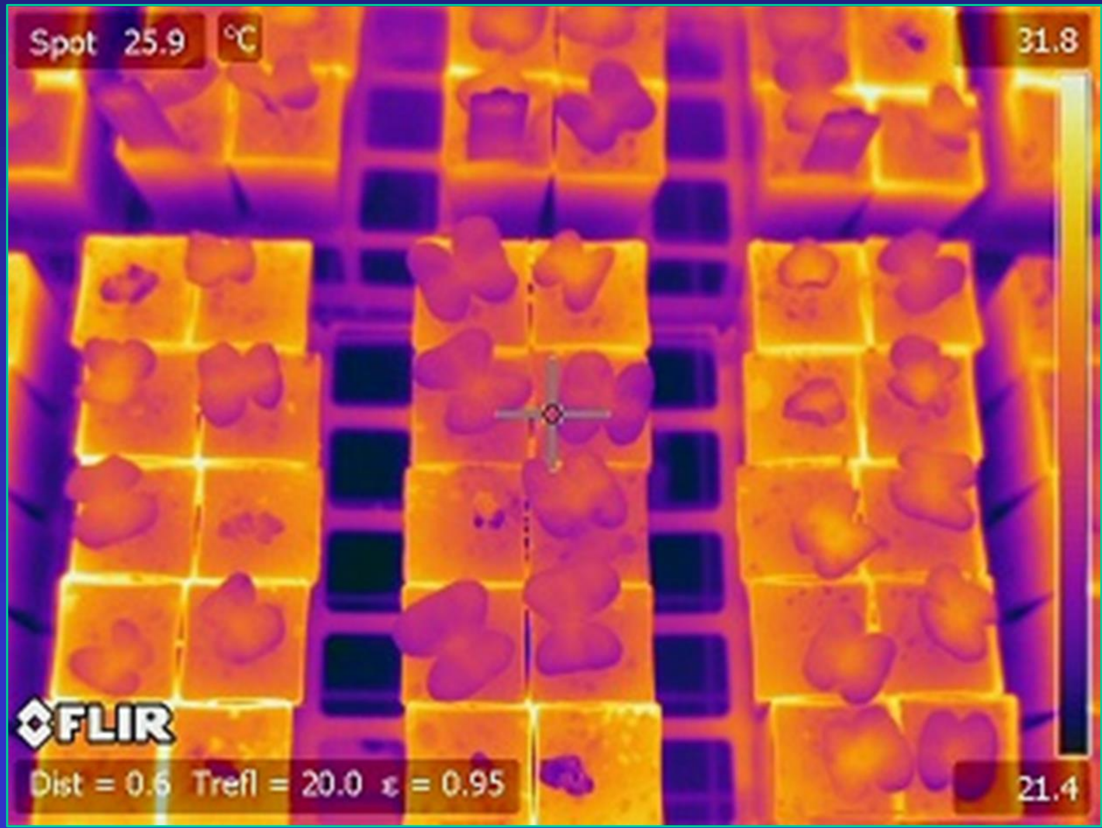
MRI allows the 3D geometry of roots to be viewed just as if the plant was growing in the soil.



Spectral reflectance

Spectral reflectance is the fraction of light reflected by a non-transparent surface.

Researchers can use spectral reflectance to tell if a plant is stressed by saline soil or drought, well before it can be seen by eye.



Plant phenomics in the field

Phenomics remote sensing technology allows researchers to study plants in the field.

- Measurements can be taken on many plants at once, and over a whole growing season

Some examples of phenomics field technology are:

- Phenonet sensor network
- Phenomobile
- Phenotower
- Blimp

Phenonet sensor network

A network of data loggers collects information from a field of crops and sends it through the mobile phone network back to researchers at the lab.

Sensors include:

- far infrared thermometer
- weather station
- soil moisture sensor
- thermistor (soil temperature)

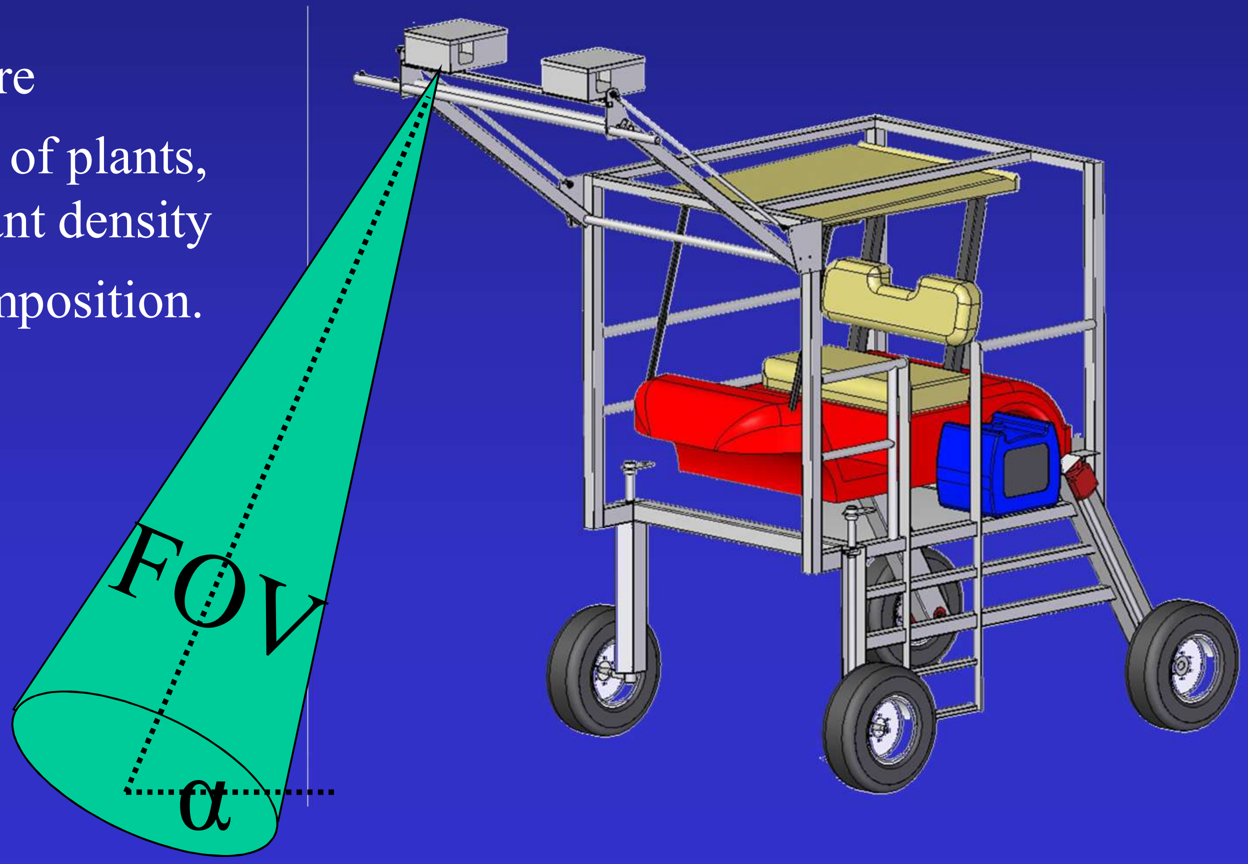
Phenomobile

The phenomobile is a modified golf buggy that moves through a field of plants, taking measurements from three rows of plants at the same time.

Phenomobile

The phenomobile carries equipment to measure:

- leaf greenness and ground cover
- canopy temperature
- volume (biomass) of plants, plant height and plant density
- crop chemical composition.



Phenotower

The phenotower is a cherry picker used to take images of crops 15 m above the ground.

Blimp

The blimp can take images of whole fields from 30 to 100 m above the ground. This allows many plants to be measured at the same time-point.

High Resolution Plant Phenomics Centre

The Centre's researchers develop new ways to discover the function of genes and to screen plant varieties for useful agricultural traits.

Researchers can grow plants in growth cabinets or in the field.

Plant Accelerator

A high-tech glasshouse contains plant conveyor systems, and imaging, robotic and computing equipment.

Research: Improving crop yields

Yearly crop yield gains have slowed to the point of stagnation.

Population growth + lack of suitable land + competition from biofuel crops + fertiliser costs + lack of water + climate change = potential global food crisis.

Phenomics projects:

- 'Supercharging' photosynthesis
- Improving wheat yield
- *Brachypodium* – the cereal 'lab rat'

‘Supercharging’ photosynthesis

Plants have two major photosynthetic mechanisms: C3 and C4. Phenomics researchers want to replace the C3 pathway of rice with a more efficient C4 mechanism.

C4 plants can concentrate carbon dioxide inside the leaf, and photosynthesise more efficiently than C3 plants, especially under:

- higher temperatures
- drought conditions
- limited nitrogen supplies.

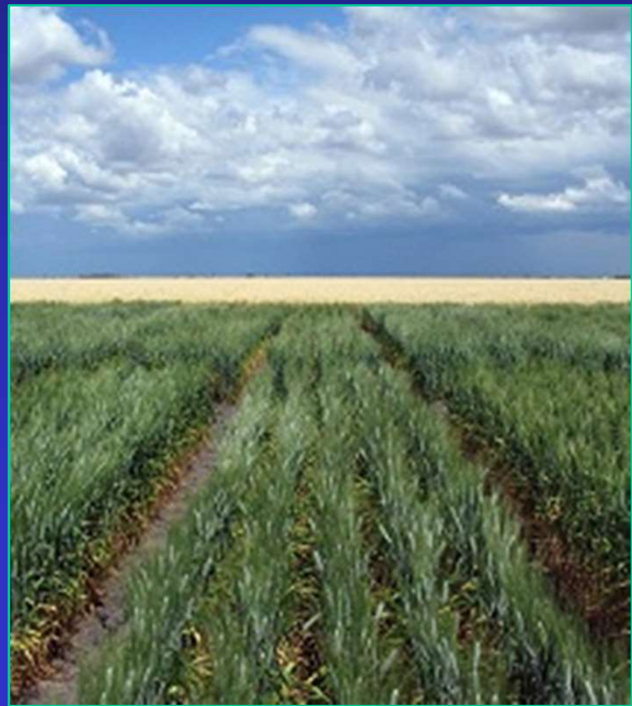
Improving wheat yield

A major limiting factor in photosynthetic performance is the inefficiency of the enzyme Rubisco.

Some plants have better Rubiscos than others.

Phenomics researchers are searching through thousands of wheat varieties for those:

- with a better-performing Rubisco and higher rates of photosynthesis
- that can grow well under nutrient deficiency, drought and salinity.



Brachypodium – the cereal ‘lab rat’

Phenomics researchers are using a small wild grass called *Brachypodium distachyon* as a wheat ‘lab rat’.

- Its entire genome is known
- It has many genes in common with wheat.

Researchers are studying root formation in *Brachypodium* to speed up understanding of wheat roots.

Research: Crops to cope with climate change

Climate change is predicted to make crop growing conditions tougher in the future.

Phenomics researchers are developing:

- drought-tolerant wheat
- salt-tolerant wheat and barley.

Drought-tolerant wheat

Crops use different amounts of water at different growth stages and under different environmental conditions.

To breed drought-tolerant wheat, researchers have to study performance in the field over a whole growing season.

Phenomics remote sensing technology can measure:

- if plants are stressed by drought conditions
- canopy temperature
- weather and soil data.

Salt-tolerant wheat and barley

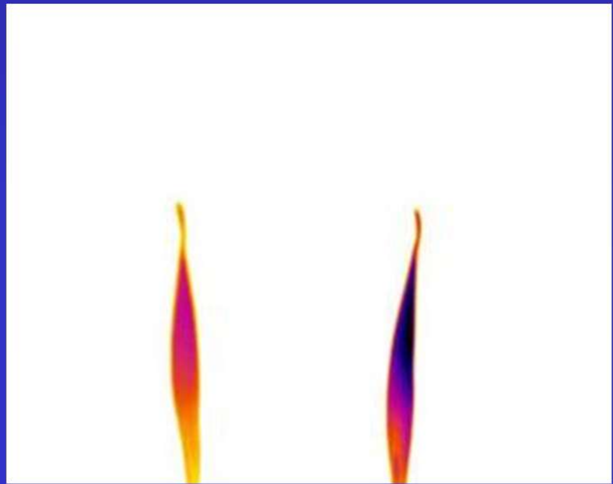
Researchers are screening wheat and barley growing in saline conditions for salt-tolerant varieties.

Plants grown in salty soil close their stomata to reduce water loss. This:

- slows photosynthesis and reduces yield
- heats the leaves.

Infrared cameras can quickly identify which plants are cooler, and are keeping their stomata open.

Plant grown in salty soil (warmer)



Plant grown in normal soil (cooler)

Conclusions

- Nitrogen and other resources are important and we need to increase their efficiency.
- New methods of screening plant genotypes like phenotyping will help develop better cultivars in a shorter time.


THANK YOU!