與Transceptor CHL1共舞的科學路

My scientific journey with transceptor CHL1

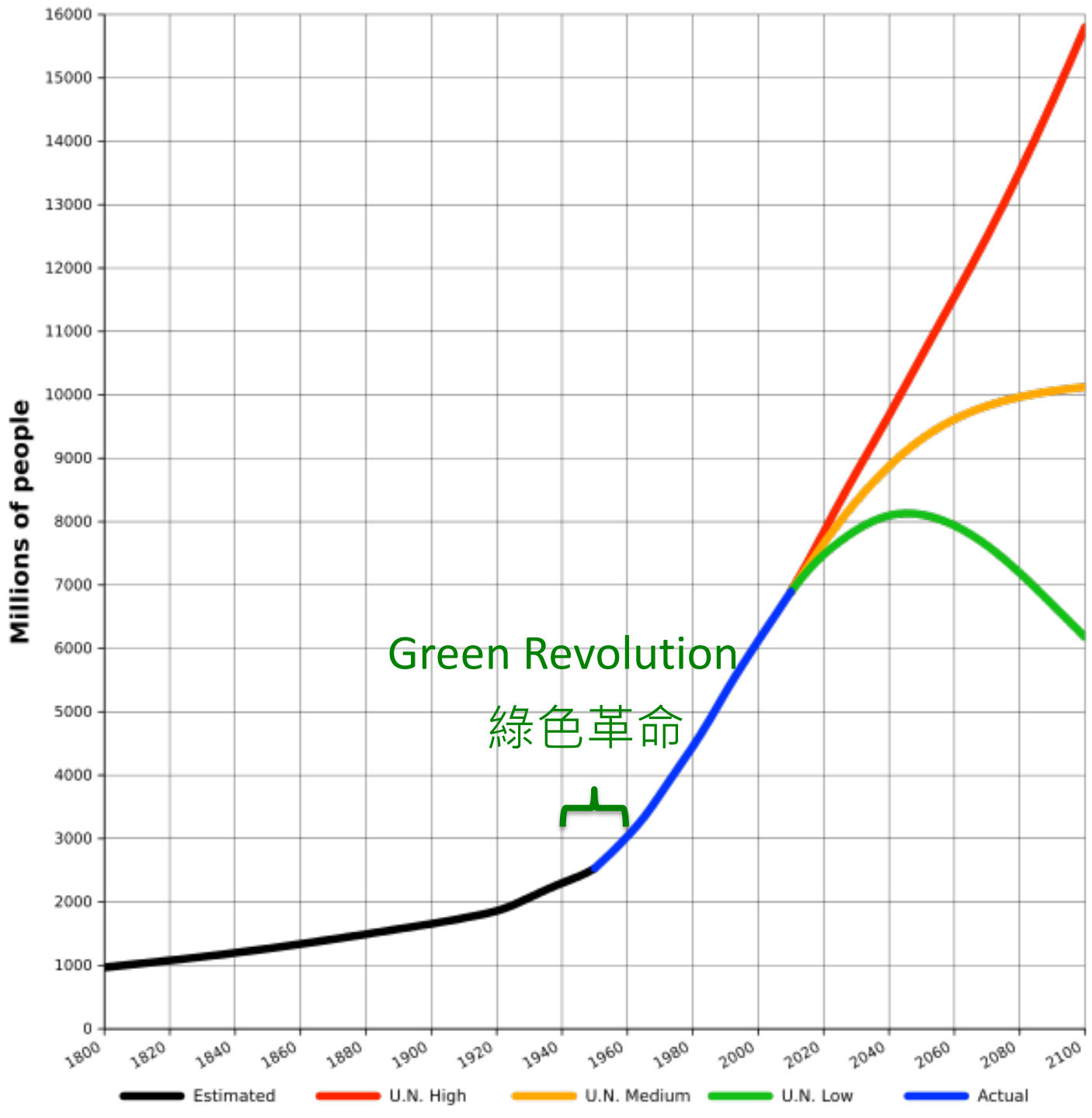
The stories behind the science

蔡宜芳

Yi-Fang Tsay

中央研究院分子生物研究所


為什麼我花了三十幾年的歲月在關愛硝酸鹽nitrate?
Why study nitrate?
氮肥的美麗與哀愁

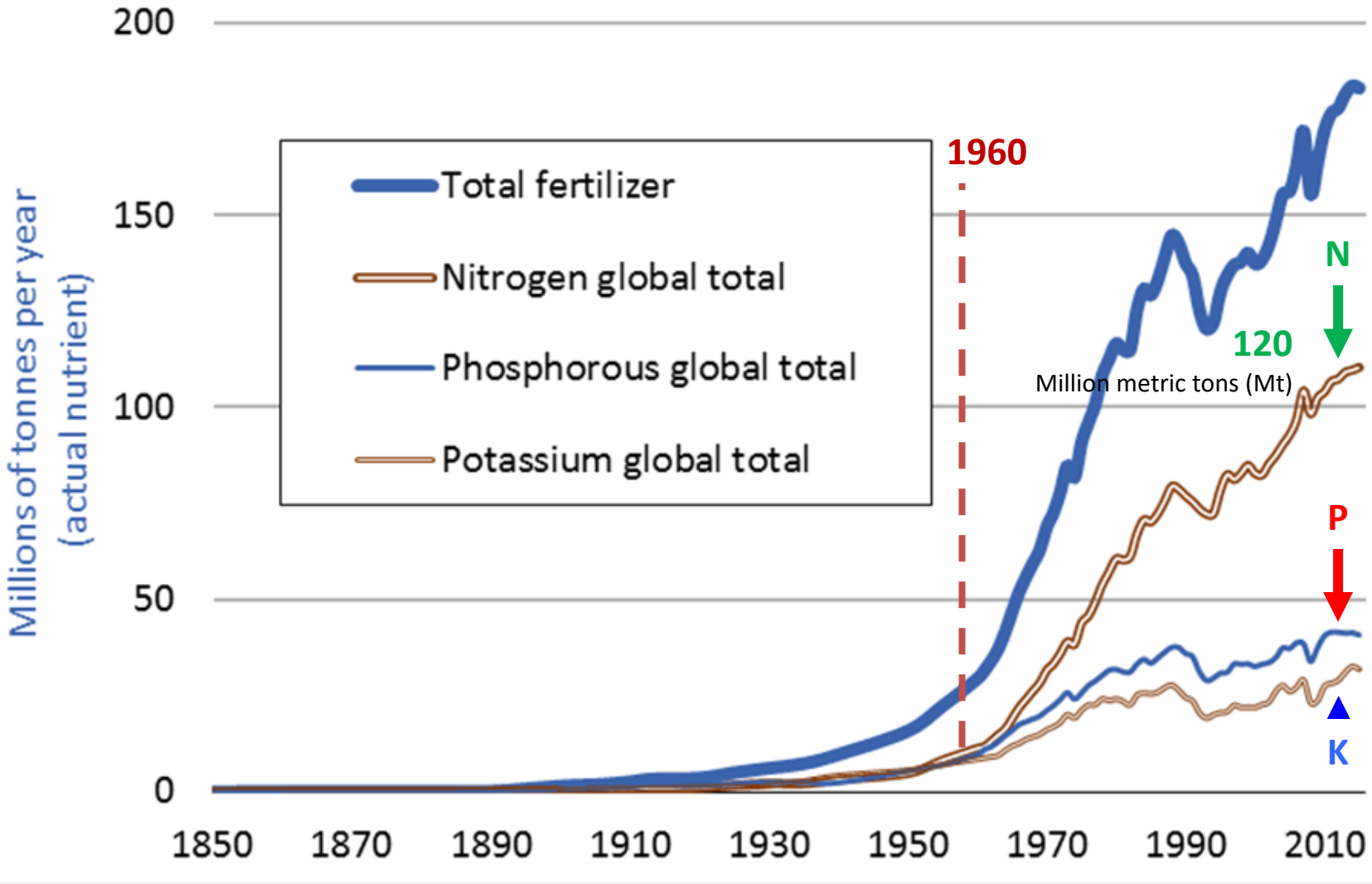

Millions of people
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1800
1820
1840
1860
1880
1900
1920
1940
1960
1980
2000
2020
2040
2060
2080
2100
Green Revolution
綠色革命
Estimated
U.N. High
U.N. Medium
U.N. Low
Actual

綠色革命
Green Revolution

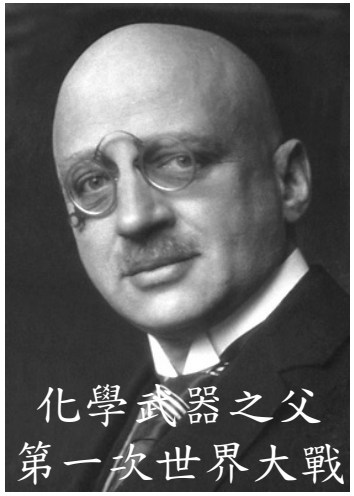
- 1940 ～ 1970
- 拯救上億人口，不受饑餓威脅。

- 高產量的新品種
- 灌溉系統的建立
- 管理技術的現代化
- 肥料及殺蟲劑的使用

N fertilizer enhances crops production



Haber-Bosch Process 哈柏法

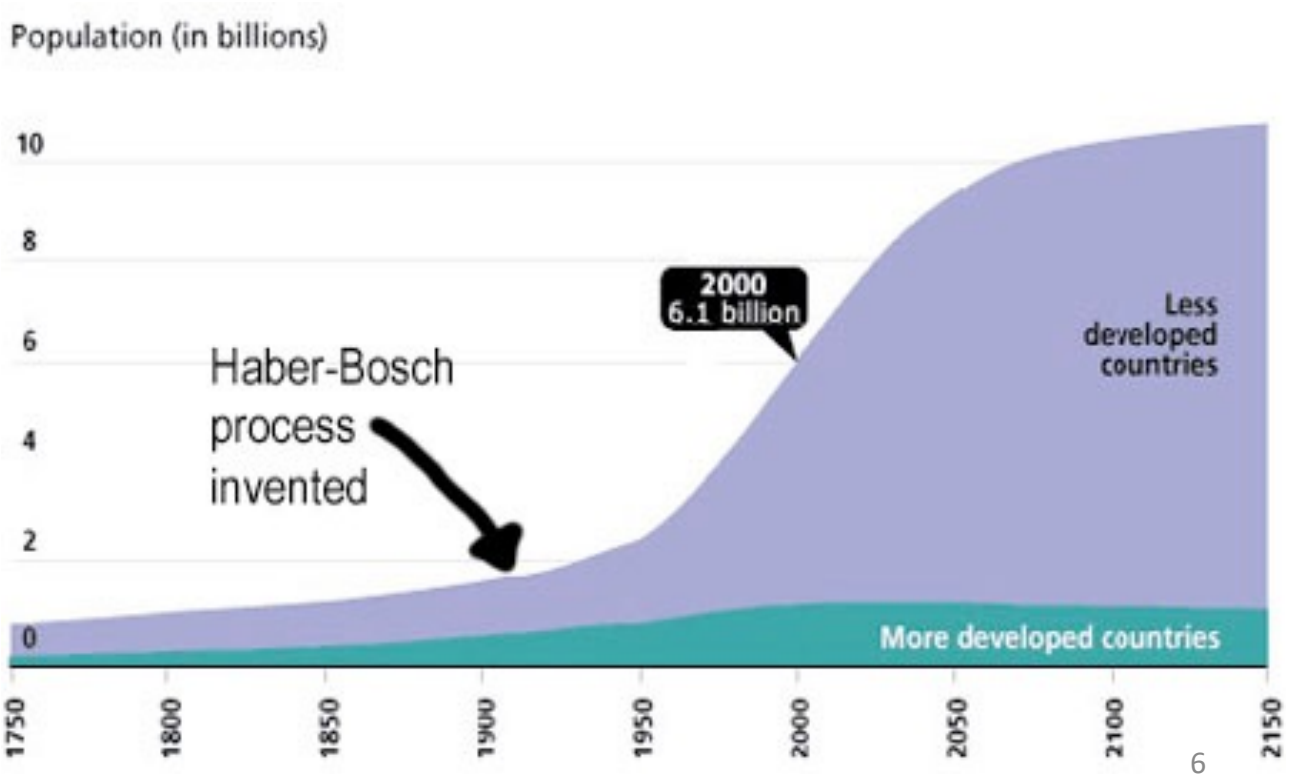


Fritz Haber
(Nobel prizes in 1918)

Carl Bosch
(Nobels in 1931)

$$N_2 + 3\ H_2 \rightarrow 2\ NH_3$$

- Half of N in our body comes from this process




為什麼我花了三十幾年的歲月在關愛硝酸鹽nitrate?
Why study nitrate?
氮肥的美麗與哀愁

N fertilizer synthesis consumes 2% of energy supply worldwide

Environmental problems

- Only 30%~50% N fertilizer applied is utilized by crops
- The remainder lead to generate green house gas nitrous oxide N_2O which is 300X more potent than CO_2.
- Eutrophication

Eutrophication 優養化

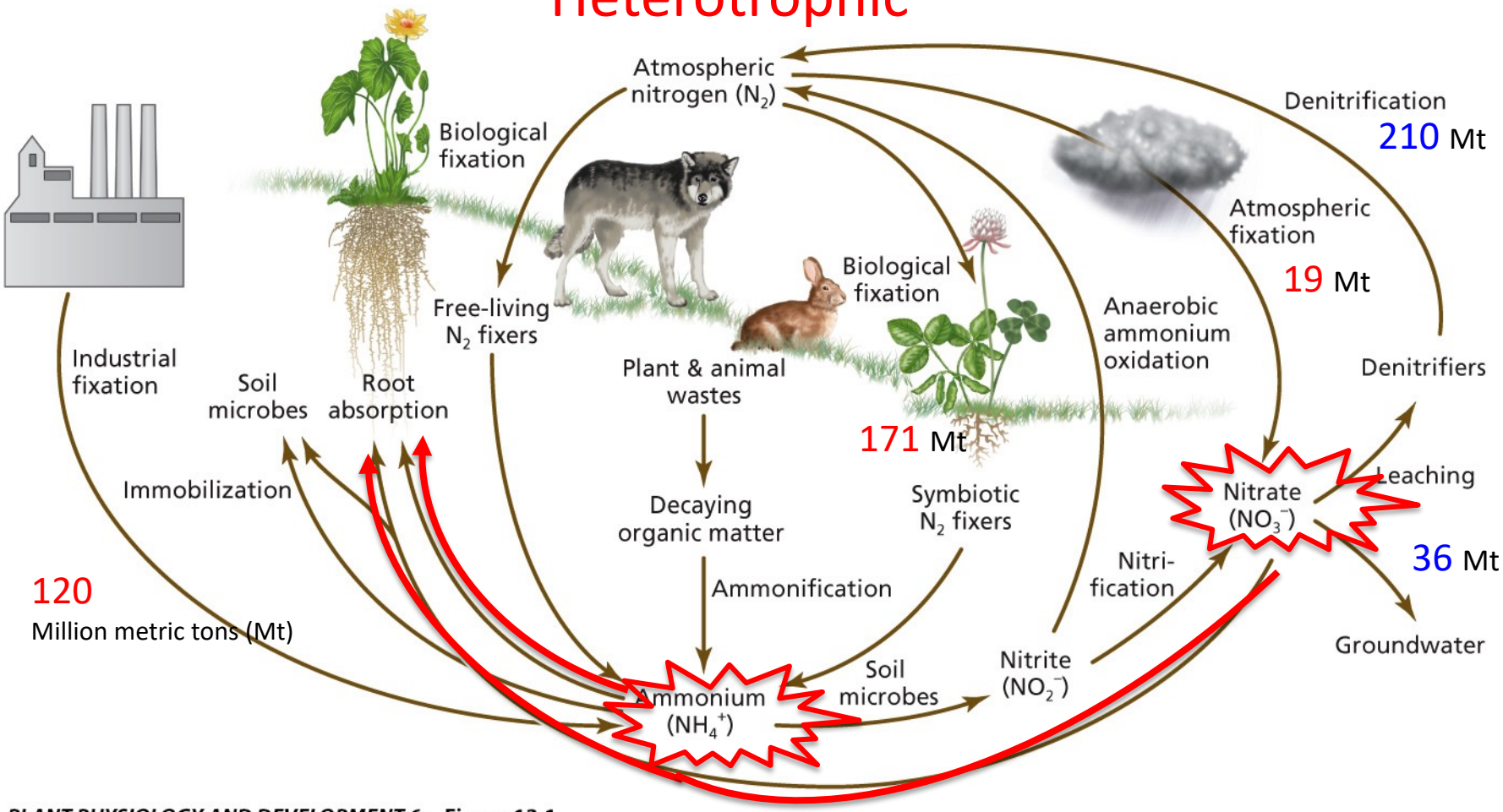
藻華

NASA

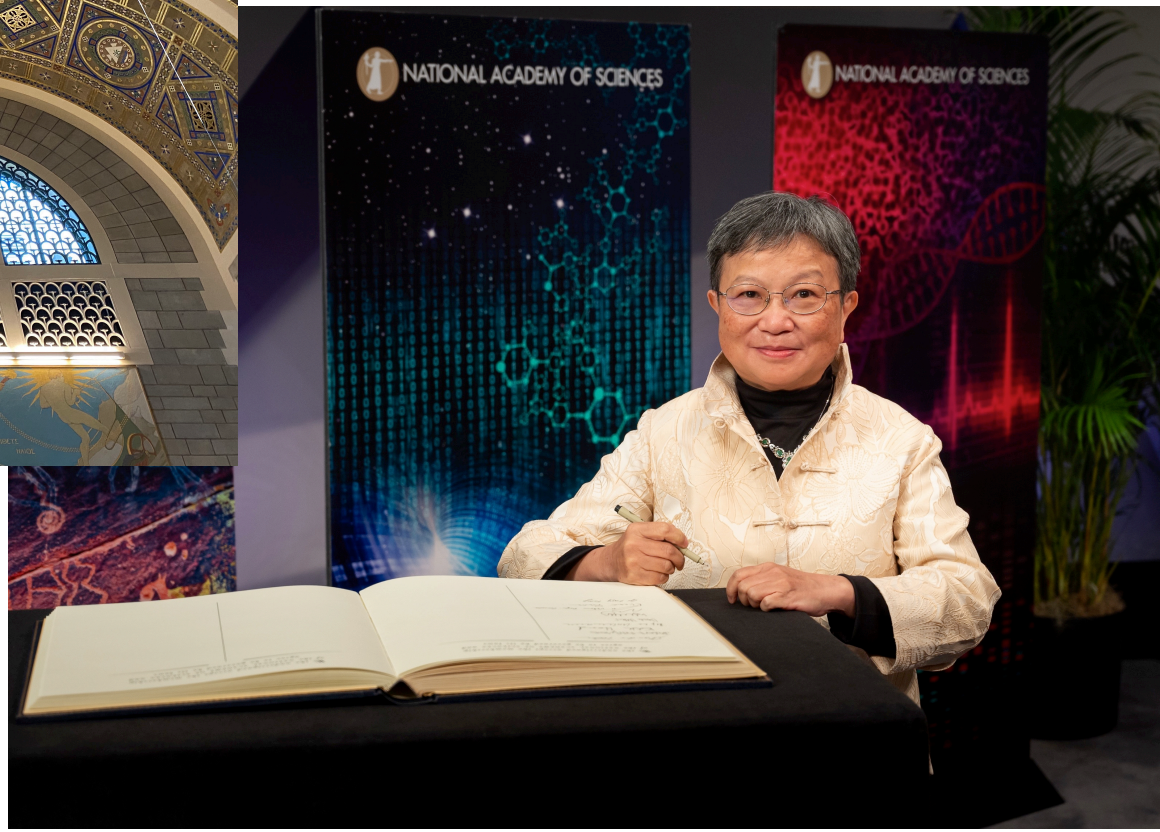
N
Autotrophic

N
Heterotrophic

Why study nitrate?



***PLANT PHYSIOLOGY AND DEVELOPMENT 6e*, Figure 13.1**

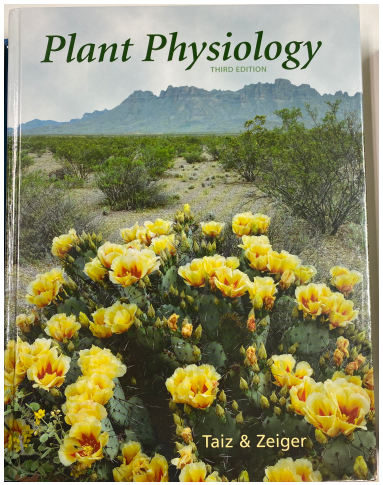


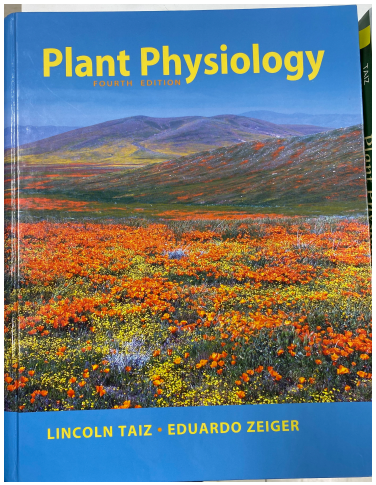
congratulations, thats so great to see that you are a NAS fellow! Happy to see that after going through tough times, you received this honor!!!

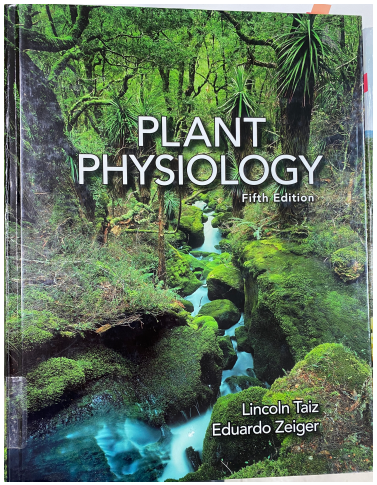
-Wolf Frommer

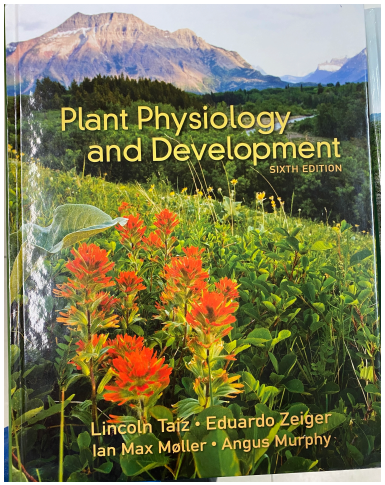
1st, 1991

2nd, 1998









3rd, 2002

4th, 2006

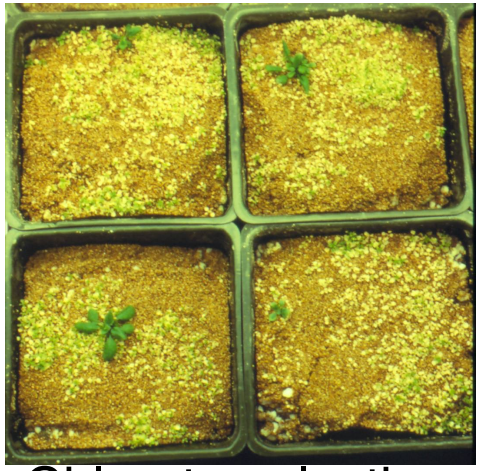
5th, 2010

6th, 2015

CHL1: Nitrate transporter
1993

Identification of the first nitrate transporter CHL1 (NRT1.1)

- 天時、地利、人和
- Nitrate uptake mutant *chl1* was isolated by Feenstra group in Netherlands in 1971
- Kenneth A Feldmann developed the T-DNA tagging technique.
- 1990-1993, UCSD



Chlorate selection

Cell, Vol. 72, 705–713, March 12, 1993, Copyright © 1993 by Cell Press

The Herbicide Sensitivity Gene *CHL1* of Arabidopsis Encodes a Nitrate-Inducible Nitrate Transporter

Yi-Fang Tsay,* Julian I. Schroeder,*
Kenneth A. Feldmann,† and Nigel M. Crawford*
*Department of Biology
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University of California, San Diego
La Jolla, California 92093-0116
†Department of Plant Sciences
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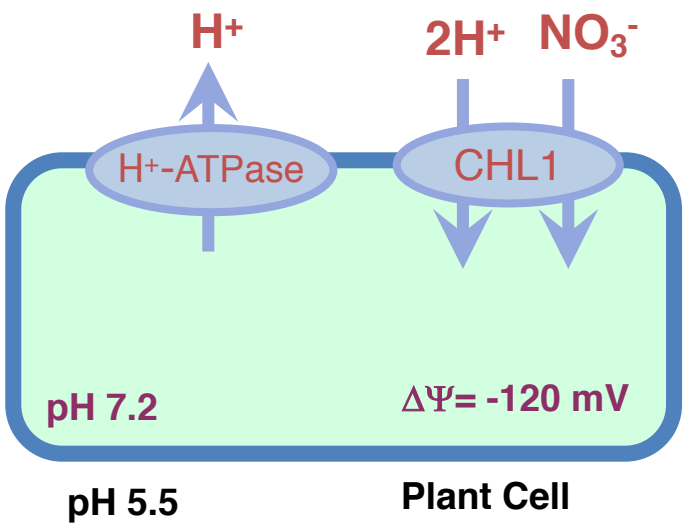
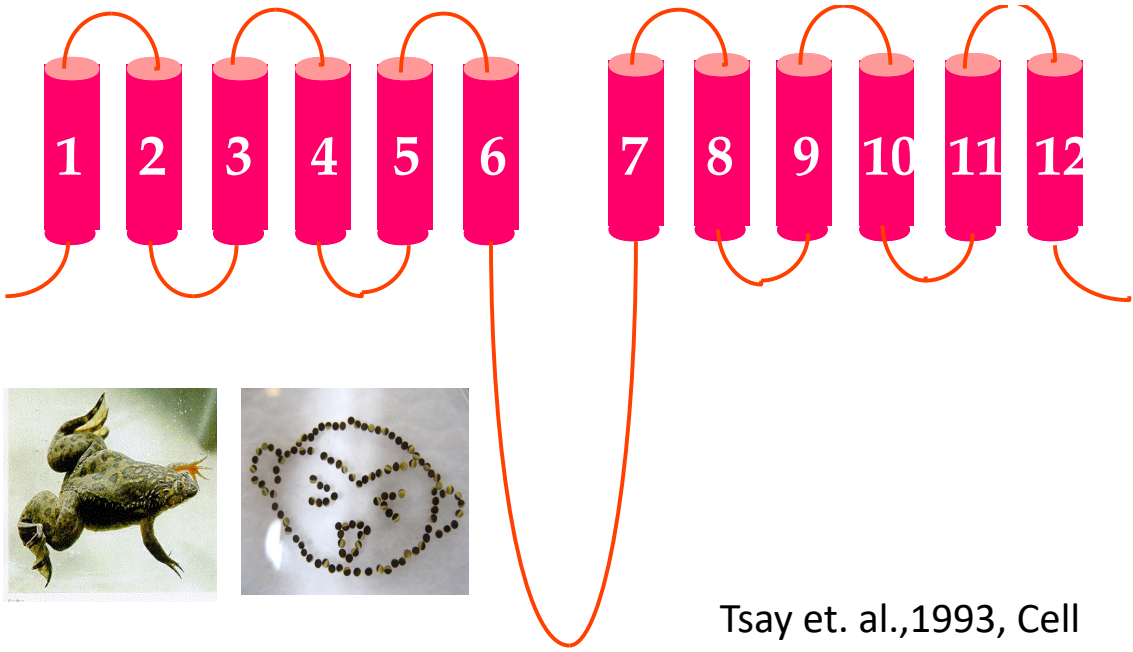
Nigel Crawford

Julian Schroeder
(Ervin Neher's PhD student)



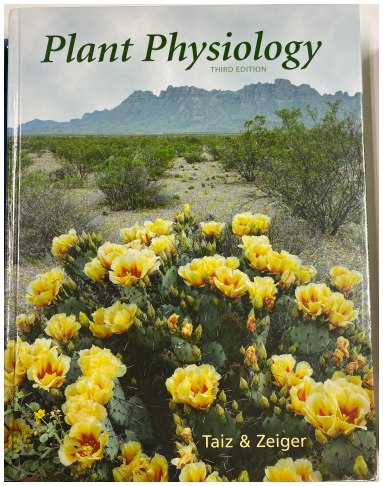


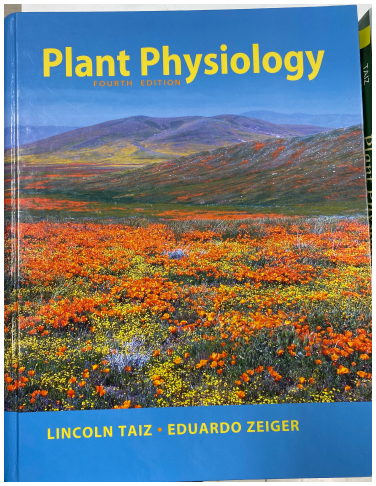
Tsay et. al.,1993, Cell

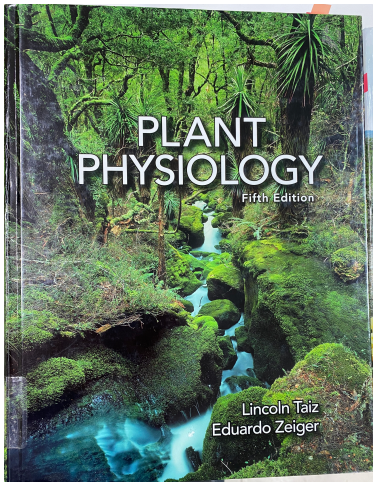
開啟了利用分子生物學來探究氮肥吸收利用之門

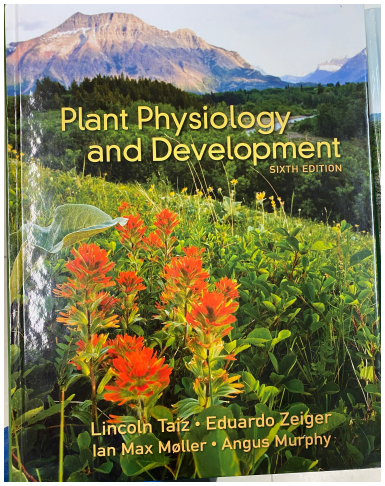
1st, 1991

2nd, 1998









3rd, 2002

4th, 2006

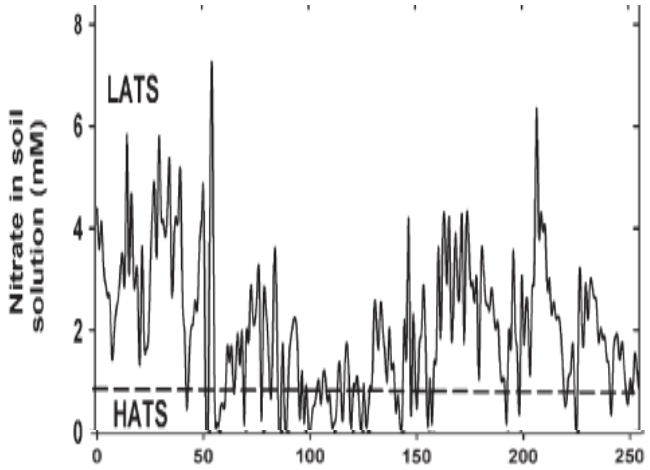
5th, 2010

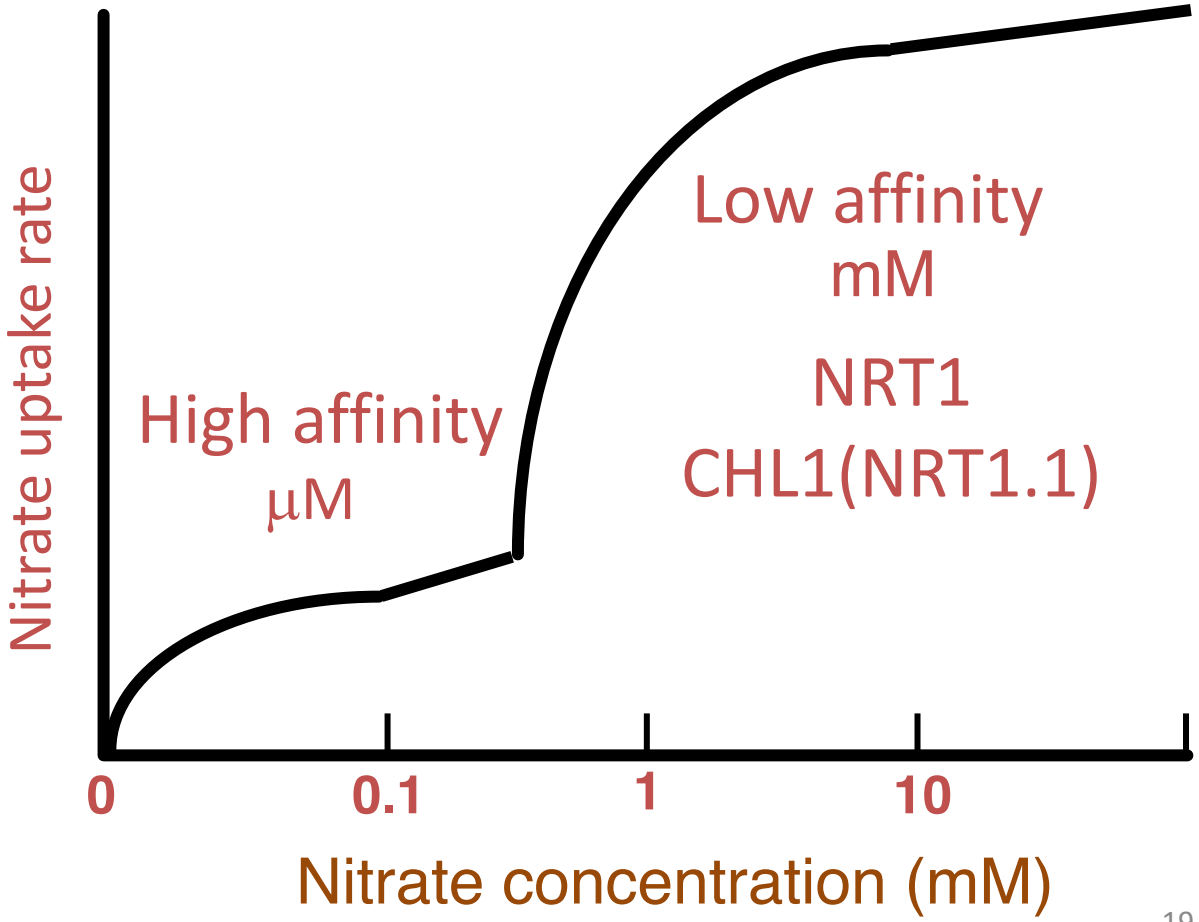
6th, 2015

CHL1: Nitrate transporter
1993

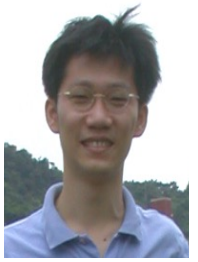
CHL1: Dual Affinity
1999, 2003

Two nitrate uptake systems





CHL1 is a dual-affinity nitrate transporter



劉坤祥

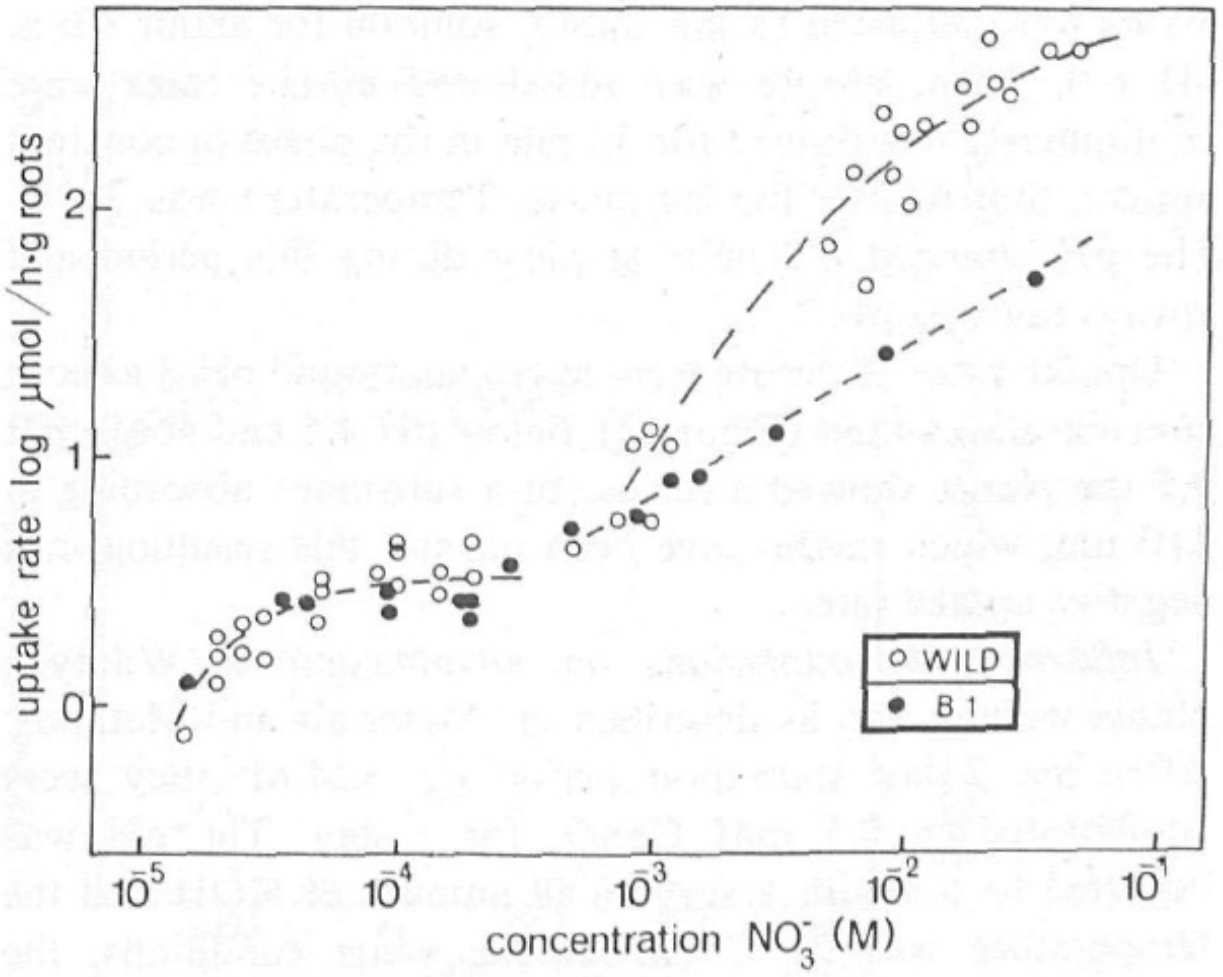
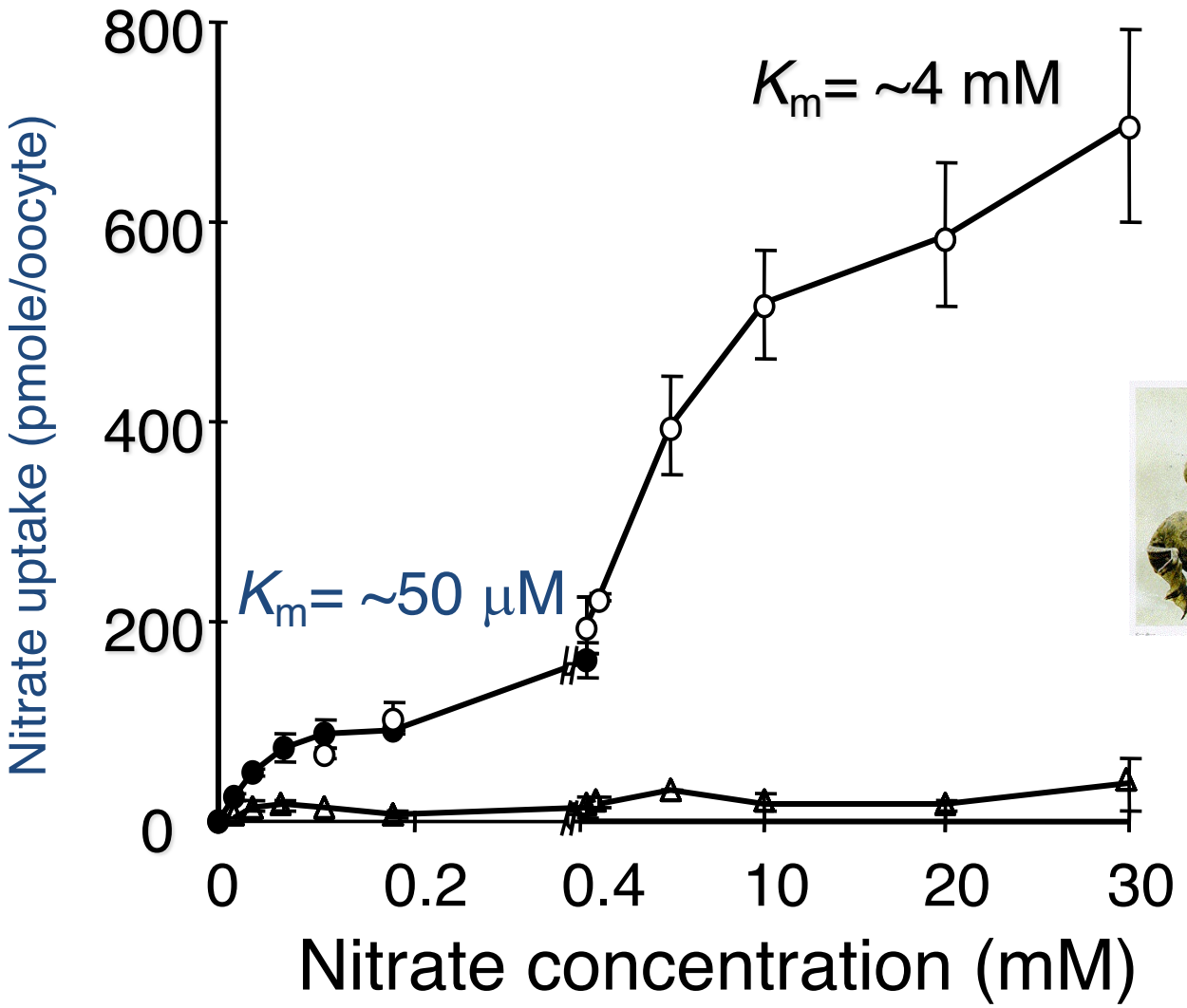
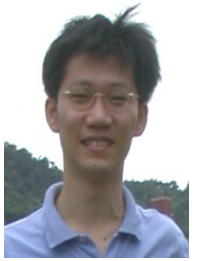


Figure 1. *Uptake rate of nitrate in relation to nitrate concentration in the wildtype and mutant B 1.* Growth and pretreatment as

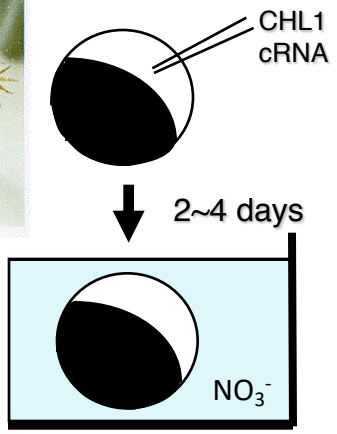
1979 Plant Physiol.

CHL1 is a dual-affinity nitrate transporter





劉坤祥



(Liu, Plant Cell, 1999)

Reprint Series
16 April 1993, Volume 260, pp. 342–344

SCIENCE

Identification of a Mobile Endogenous Tranposon in *Arabidopsis thaliana*

Yi-Fang Tsay, Mary J. Frank, Tania Page, Caroline Dean, and Nigel M. Crawford*

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 15134–15139, December 1998
Plant Biology

The *Arabidopsis* CHL1 protein plays a major role in high-affinity nitrate uptake

RONGCHEN WANG, DONG LIU, AND NIGEL M. CRAWFORD*

Department of Biology, University of California at San Diego, La Jolla, CA 92093-0116

Edited by Maarten Koornneef, Wageningen Agricultural University, Wageningen, The Netherlands, and approved October 5, 1998 (received for review July 1, 1998)

The Plant Cell, Vol. 11, 865–874, May 1999, www.plantcell.org

CHL1 Is a Dual-Affinity Nitrate Transporter of Arabidopsis Involved in Multiple Phases of Nitrate Uptake

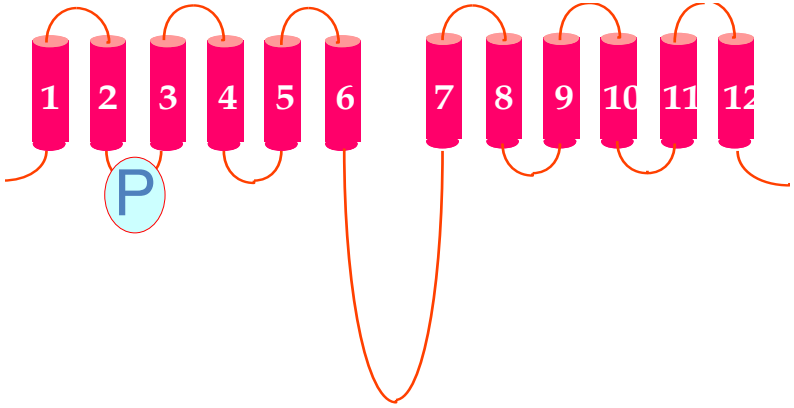
Kun-Hsiang Liu,[a,b] Chi-Ying Huang,[b] and Yi-Fang Tsay[a,1]

[a] Institute of Molecular Biology, Academia Sinica, Taipei, 11529 Taiwan
[b] Department of Botany, National Taiwan University, Taipei, 10660 Taiwan

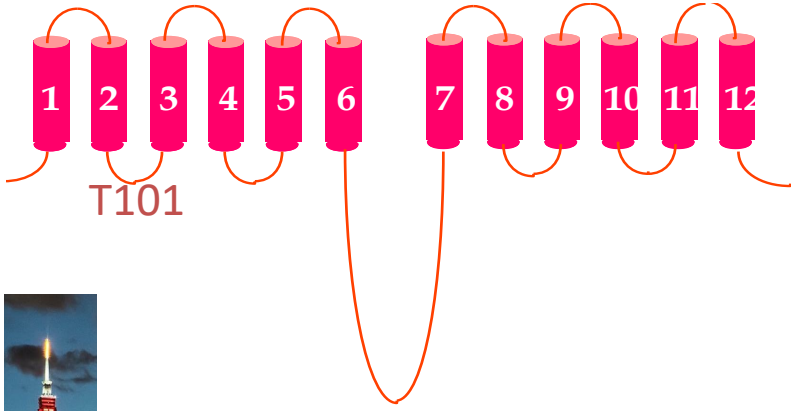
This is marathon, not a 100 M short run

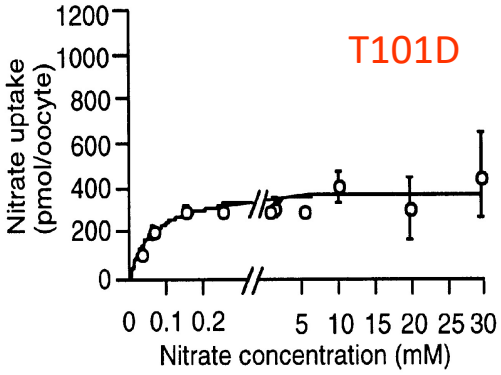
Two uptake modes of CHL1 are switched by phosphorylation

High affinity (μM)

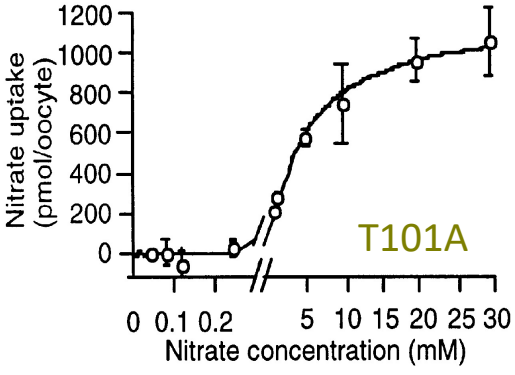


Low affinity (mM)



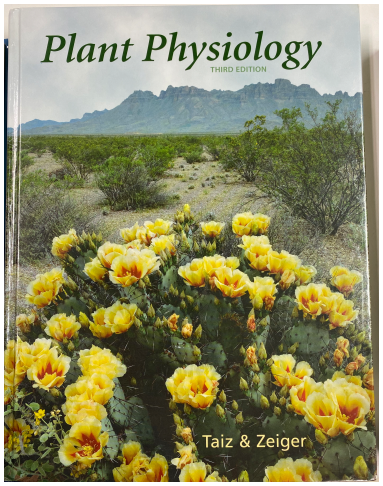


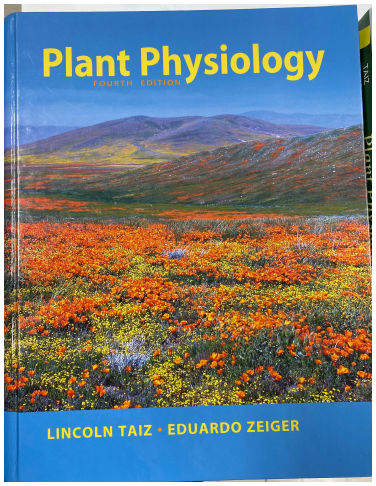
(*Liu EMBO. J*, 2003)

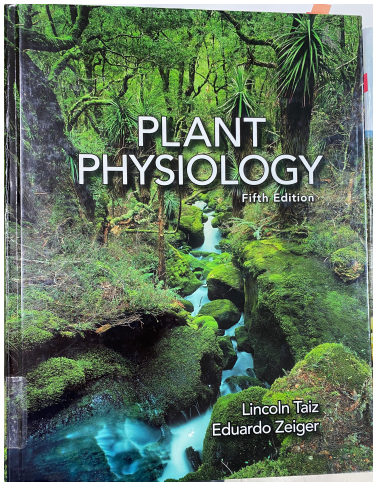


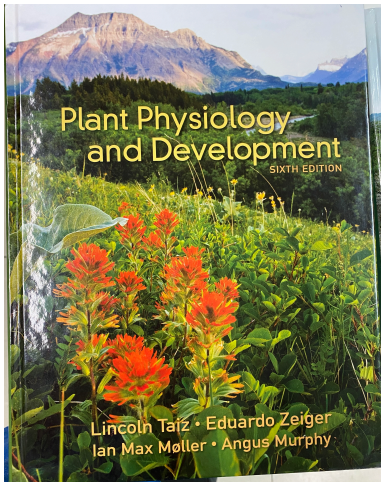
1st, 1991

2nd, 1998









3rd, 2002

4th, 2006

5th, 2010

6th, 2015

CHL1: Nitrate transporter
1993

CHL1: Dual Affinity
1999, 2003

Plant Physiol. (1997) 114: 137–144

Anthony Glass, UBC

NO_3^- and ClO_3^- Fluxes in the *chl1-5* Mutant of *Arabidopsis thaliana*[1]

Does the *CHL1-5* Gene Encode a Low-Affinity NO_3^- Transporter?

Bruno Touraine and Anthony D.M. Glass*

Laboratoire de Biochimie et Physiologie Moléculaire des Plantes, École Nationale Supérieure Agronomique de Montpellier (ENSAM), Institut National de la Recherche Agronomique, Centre National de la Recherche Scientifique, Unité de Recherche Associée 2133, 34060 Montpellier Cedex 1, France (B.T.); and Department of Botany, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4 (A.D.M.G.)

Plant Physiology®, November 2013, Vol. 163, pp. 1103–1106,

Scientific Correspondence

A Reevaluation of the Role of Arabidopsis NRT1.1 in High-Affinity Nitrate Transport[1]

Anthony D.M. Glass* and Zorica Kotur

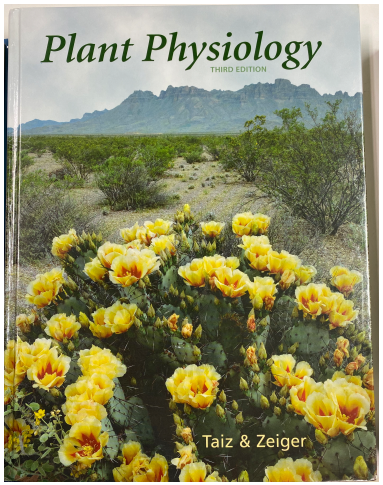
Department of Botany, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

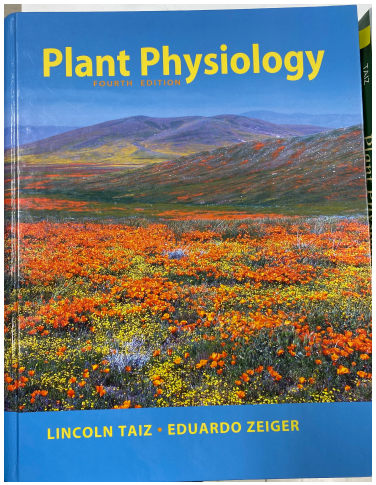
Theme 2

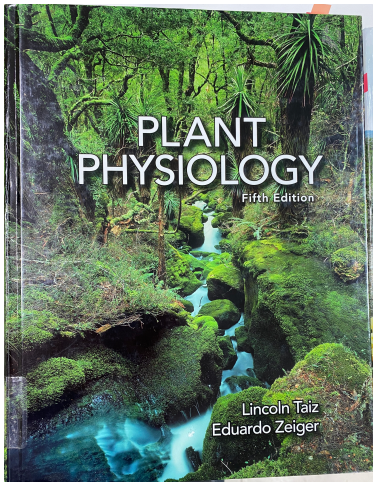
Nitrate sensing

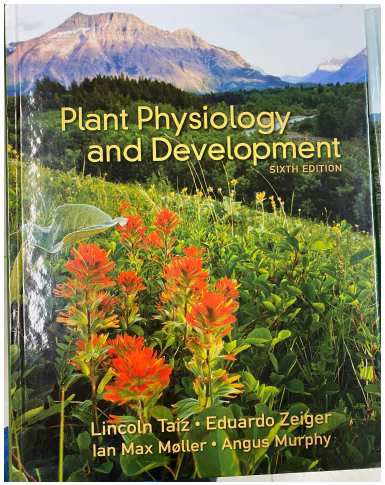
1st, 1991

2nd, 1998









3rd, 2002

4th, 2006

5th, 2010

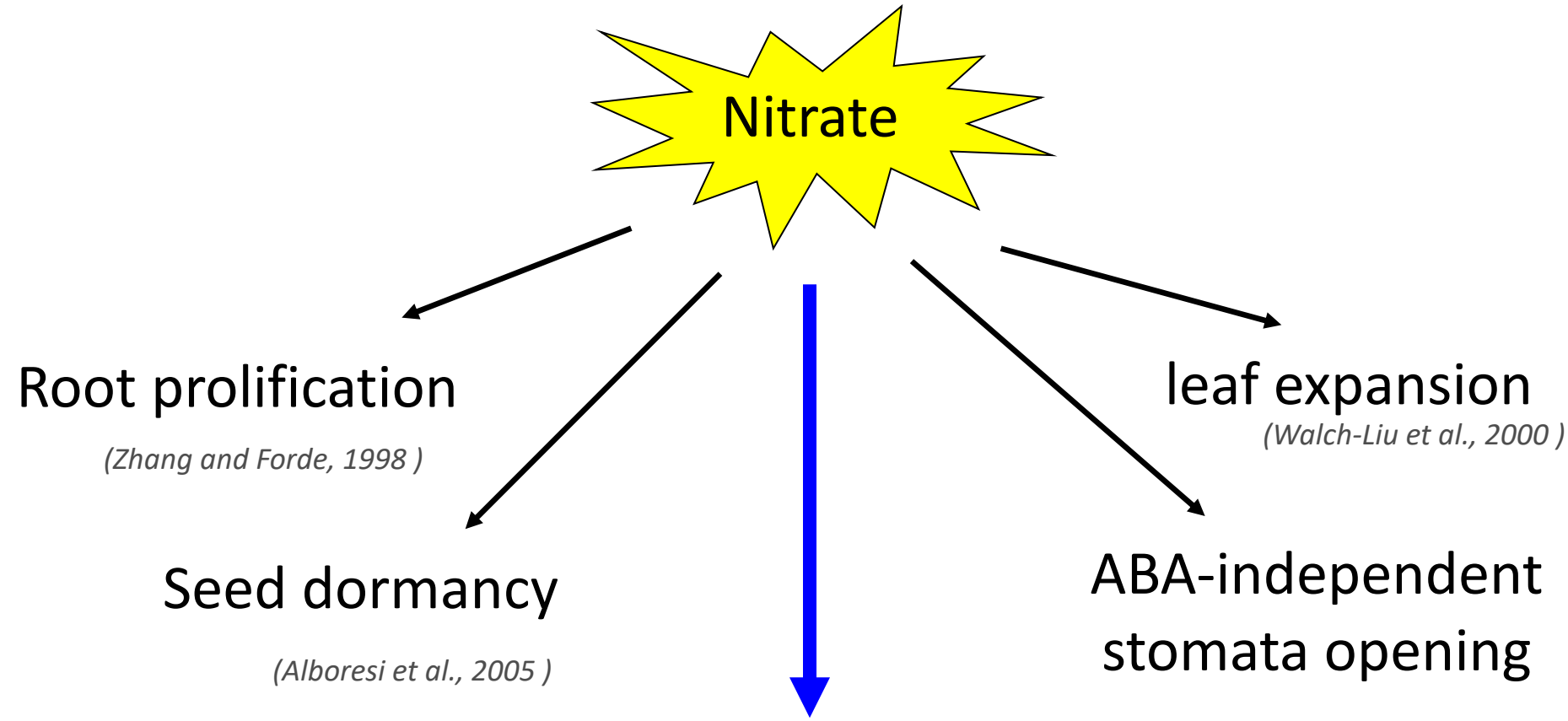
6th, 2015

CHL1: Nitrate transporter
1993

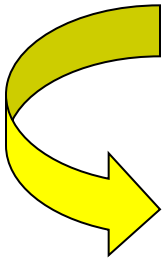
CHL1: Dual Affinity
1999, 2003

CHL1: Transceptor
2009

Nitrate also serves as a signal molecule

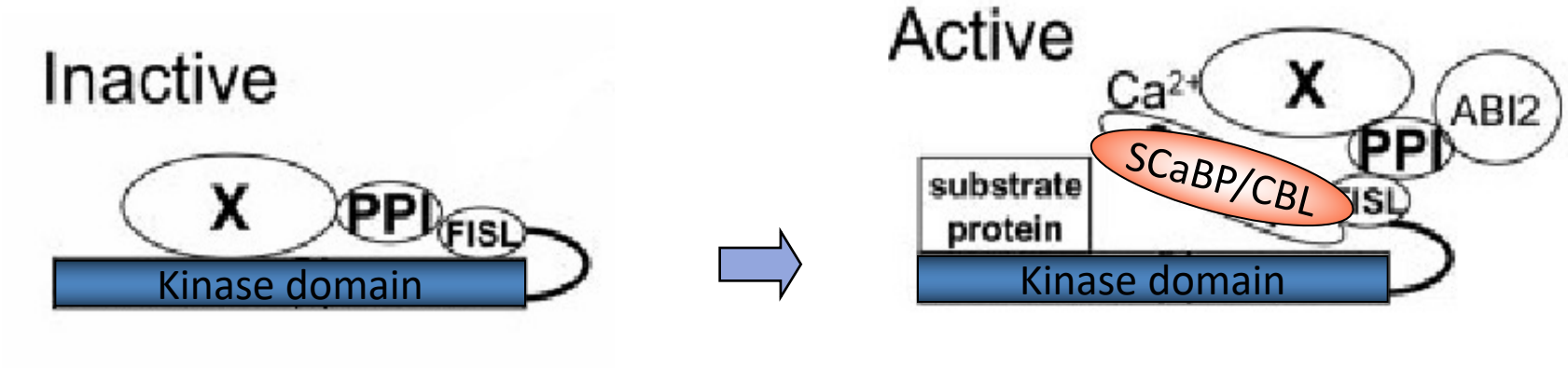


Microarray analysis of Wild type and mutant *chl1-5*



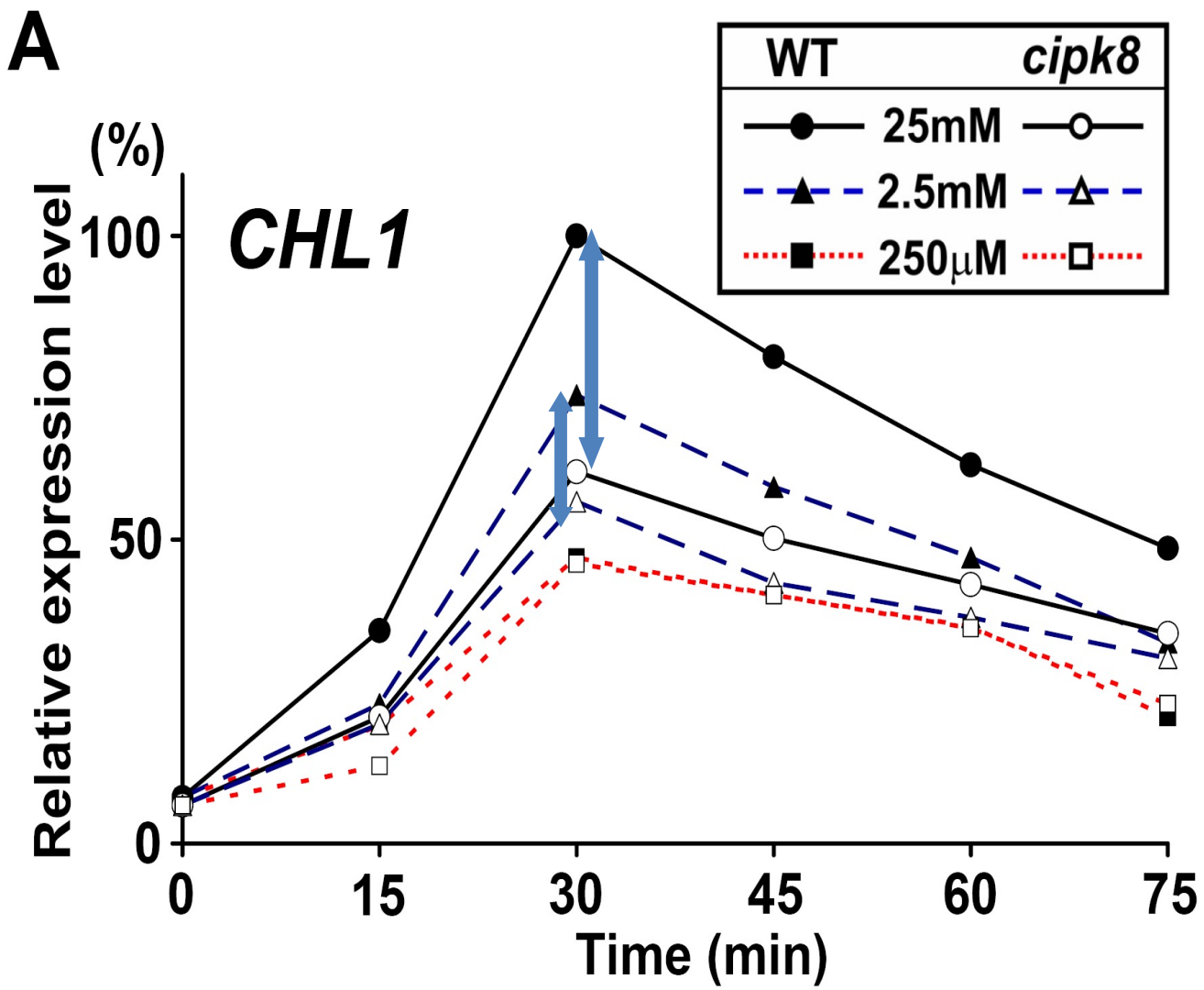
CIPK8

胡恆正



26 CIPK in Arabidopsis

CIPK8 is involved in primary nitrate response

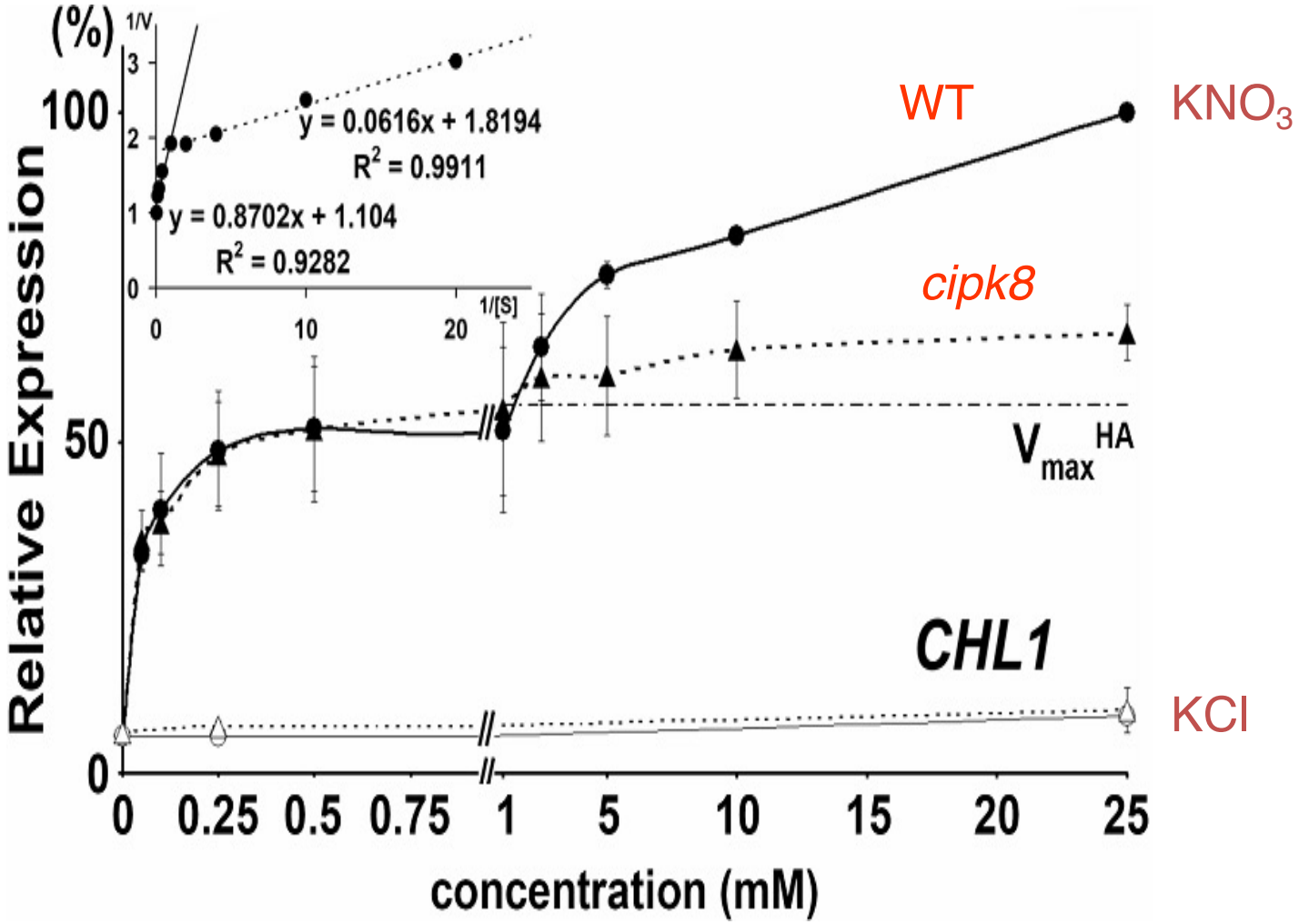


Comment we got from PNAS

Remarks to Author:

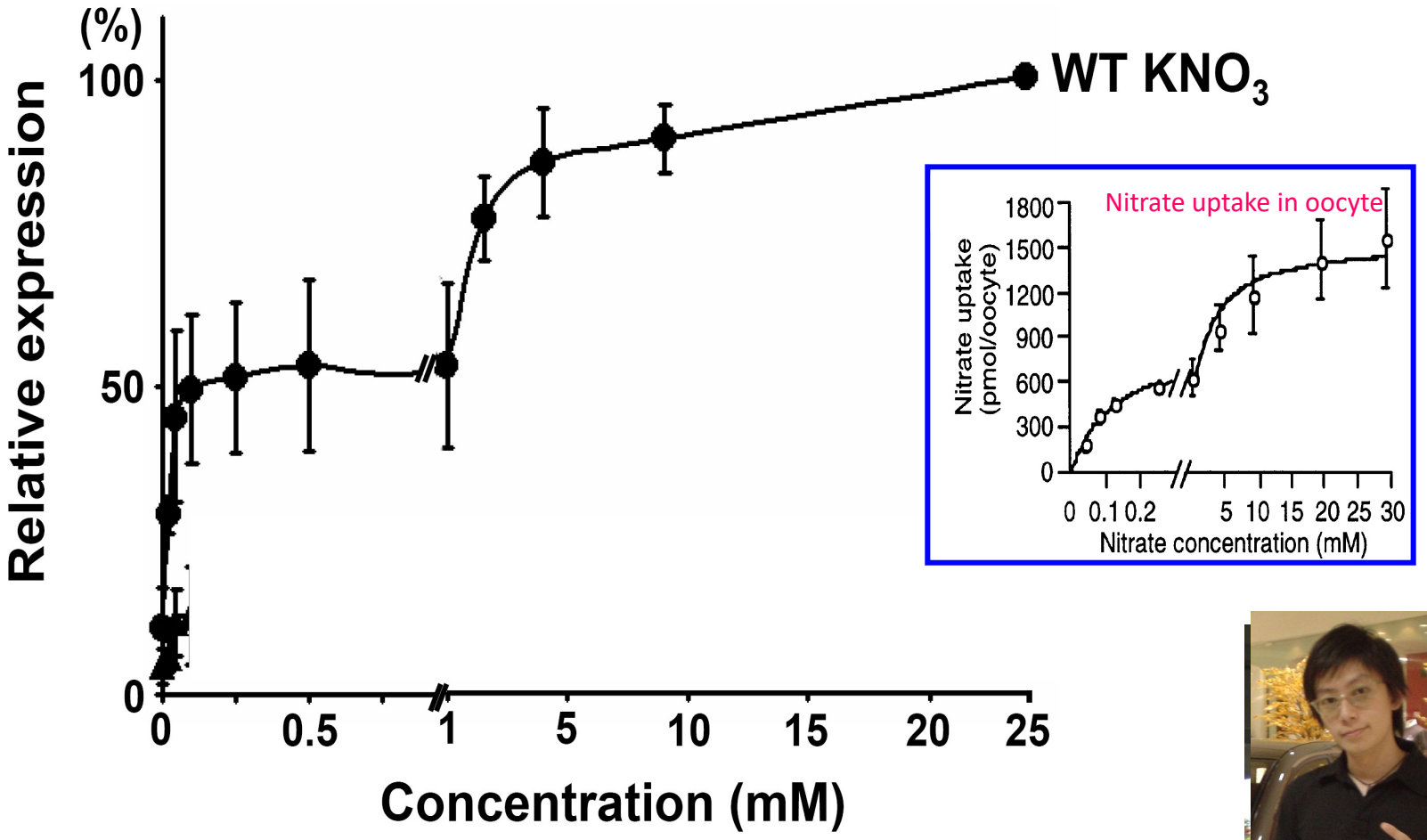
The difference in nitrate response between wild type and the CIPK8 mutant requires high levels of nitrate (25 mM) and is rather small. It is not clear that this is a nitrate primary response.

Kinetic analysis of primary nitrate response in *cipk8*



Hu, 2009 Plant J.

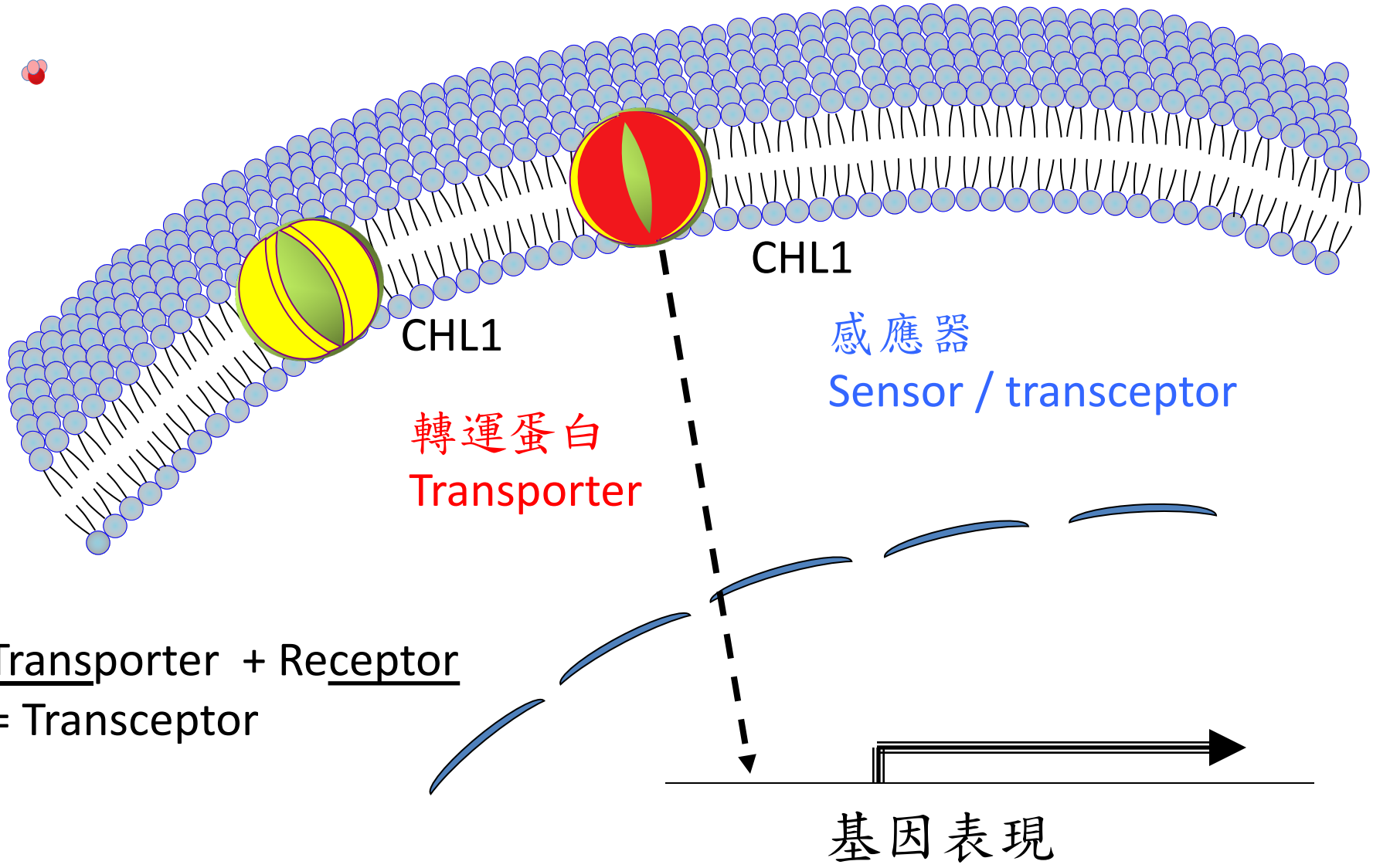
Kinetic analysis of primary nitrate response



Q-PCR analysis of *AtNRT2.1*

謝承訓

身兼數職的硝酸鹽轉運蛋白--搬運工、守門員、與傳令兵

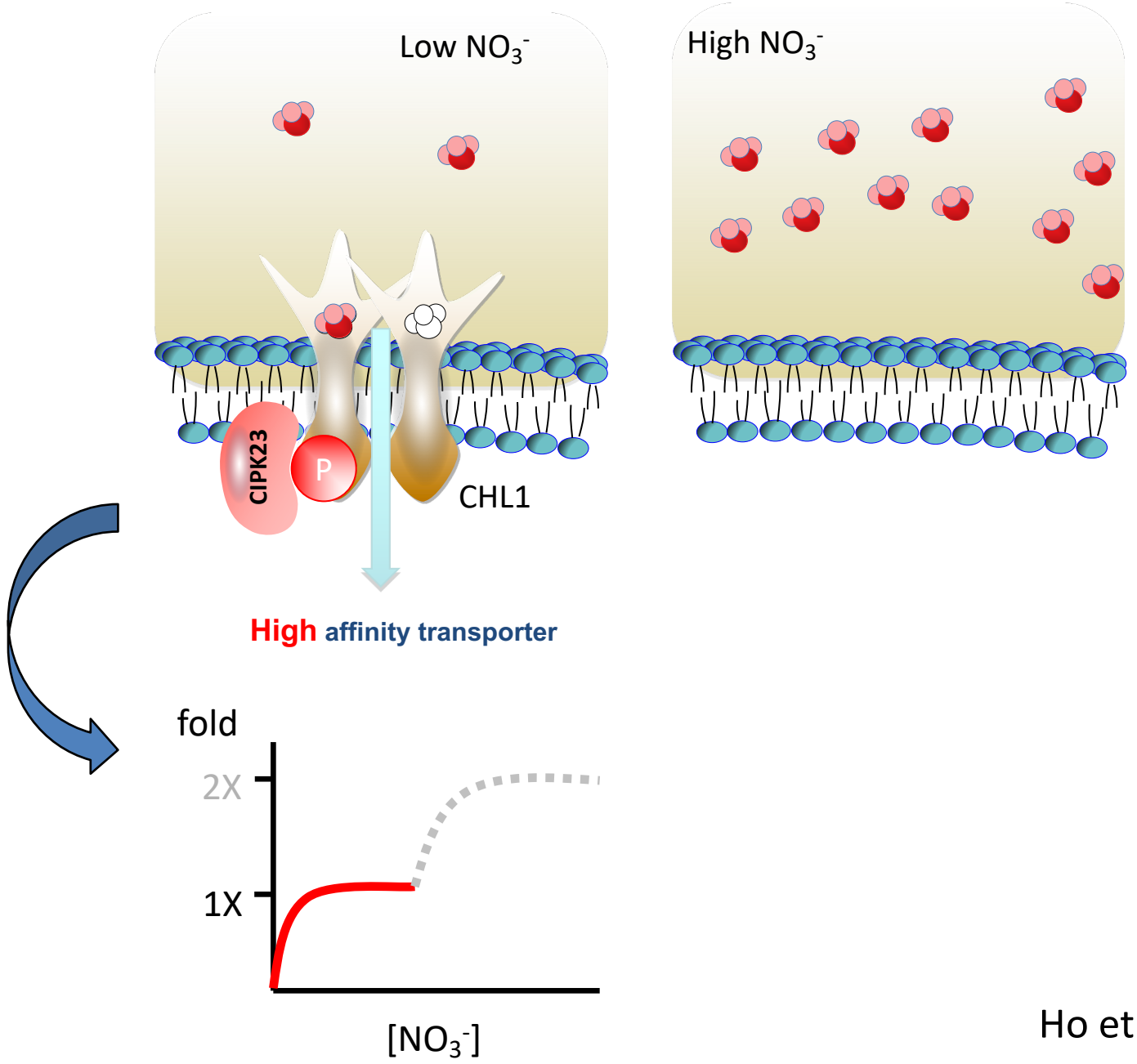


Transporter + Receptor
= Transceptor

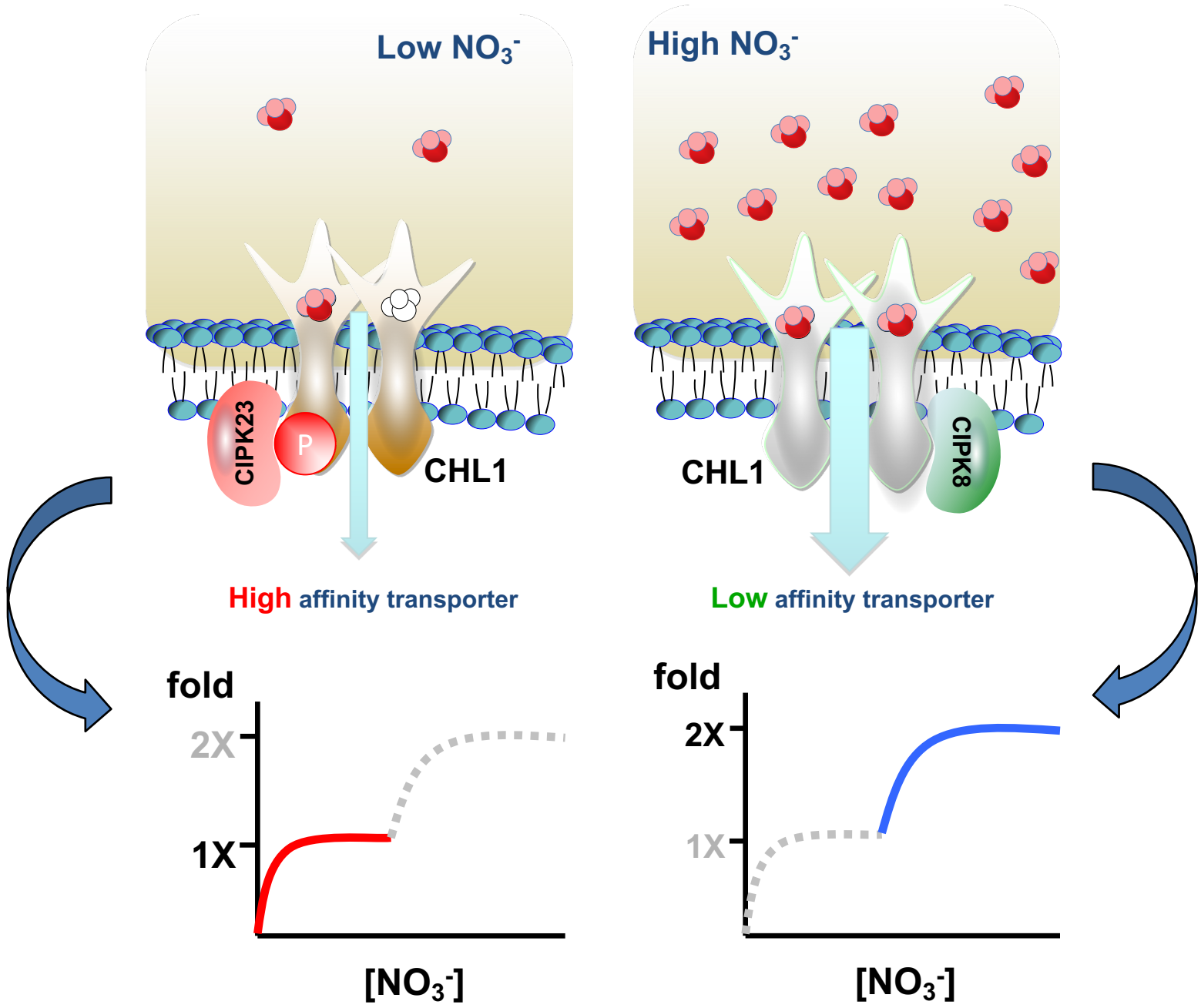
何承訓，林珊華，胡恆正，蔡宜芳 2009, Cell

植物會做數學!!

How does a sensor detect concentration changes?

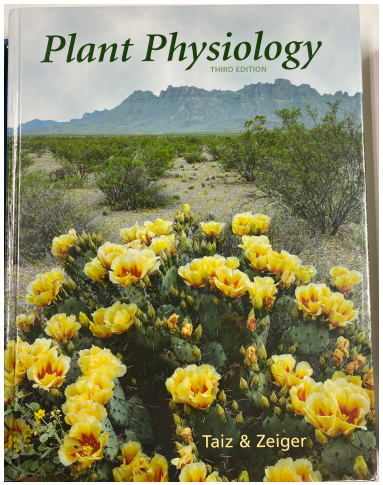


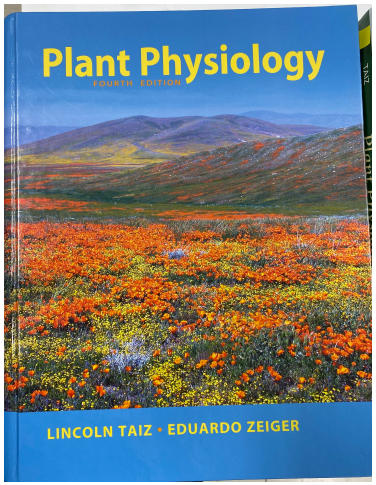
Ho et. al.,2009, Cell

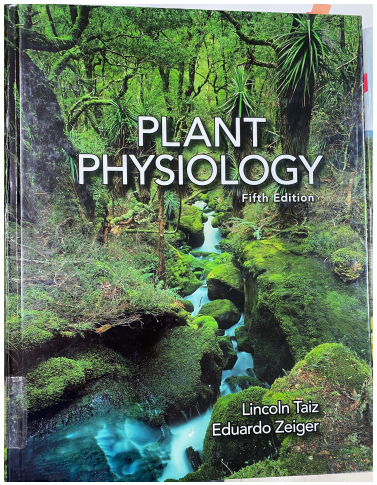

Low NO_3^-
High NO_3^-
CIPK23
P
CHL1
CHL1
CIPK8
High affinity transporter
Low affinity transporter
fold
2X
1X
$[NO_3^-]$
fold
2X
1X
$[NO_3^-]$

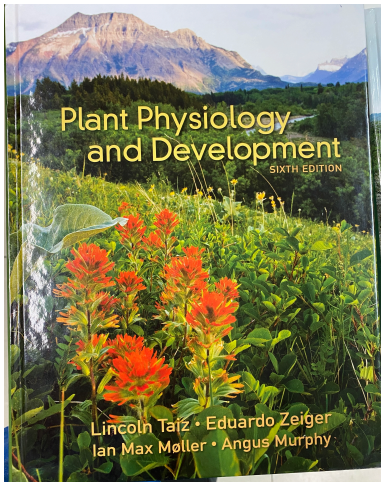
1st, 1991

2nd, 1998









3rd, 2002

4th, 2006

5th, 2010

6th, 2015

CHL1: Nitrate transporter
1993

CHL1: Dual Affinity
1999, 2003

CHL1: Transceptor
2009

Think positively and constructively about reviewer’s comments

Take Home Lesson

柳暗花明又一村

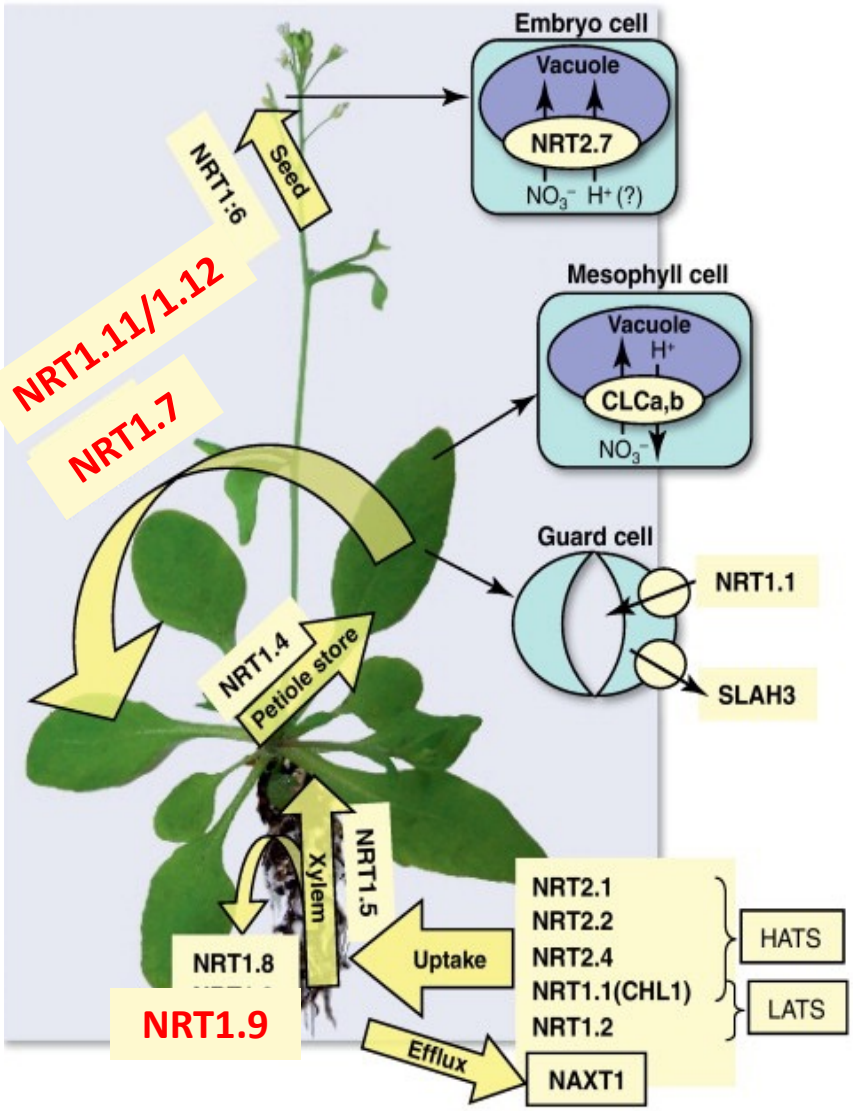
—化危機為轉機

以建設性的態度面對reviewer的批判

Theme 3

盡信書不如無書----談硝酸鹽運送的研究如何改寫教科書

～改寫教科書理論的酸甜苦辣


Embryo cell
Vacuole
NRT2.7
NO_3^- H^+ (?)
Seed
NRT1.6
NRT1.11/1.12
NRT1.7
Mesophyll cell
Vacuole
H^+
CLCa,b
NO_3^-
Guard cell
NRT1.1
SLAH3
NRT1.4
Petiole store
Xylem
NRT1.5
NRT1.8
NRT1.9
Uptake
NRT2.1
NRT2.2
NRT2.4
NRT1.1(CHL1)
NRT1.2
HATS
LATS
Efflux
NAXT1

A classical statement of nitrogen transport in text book:

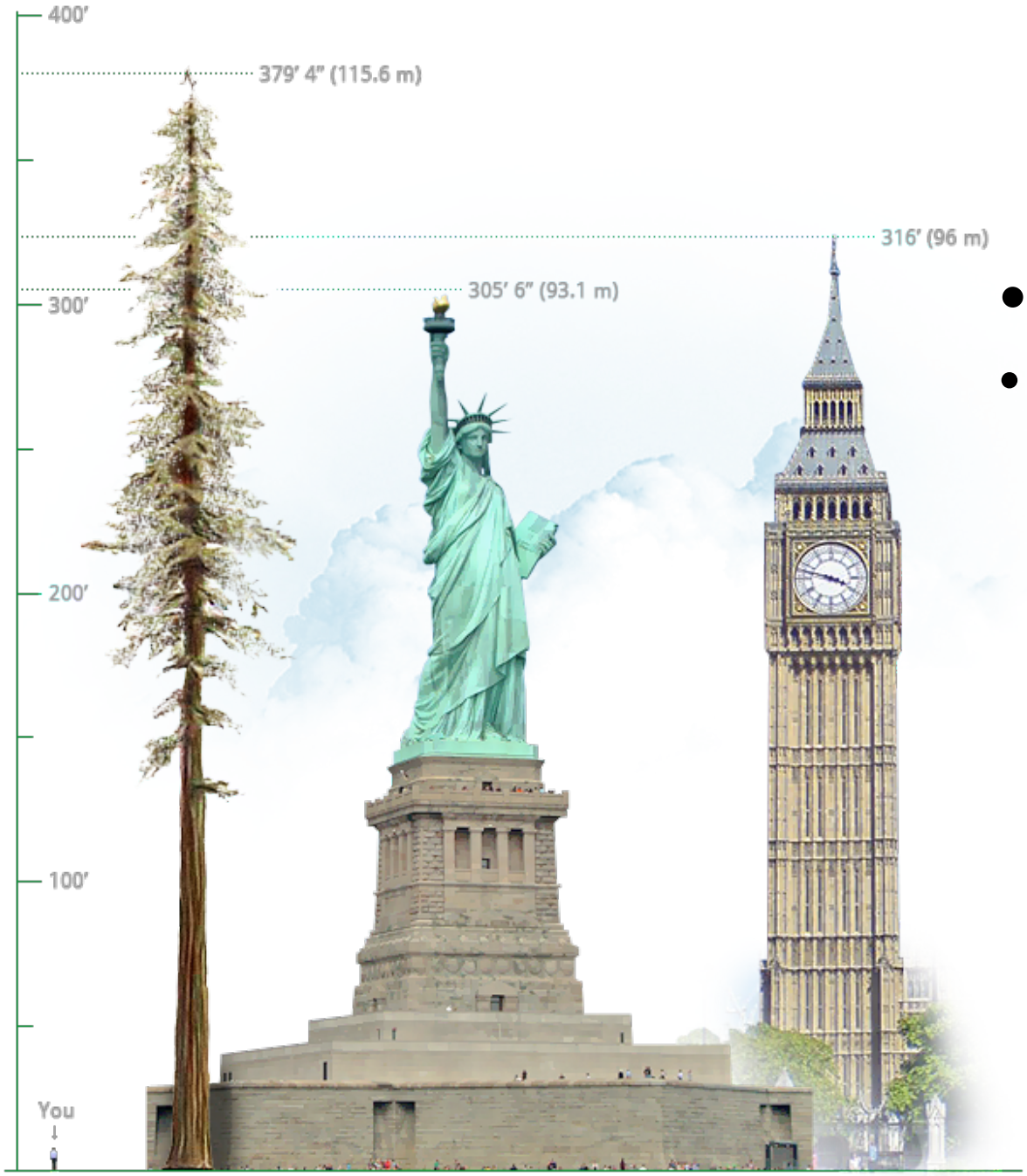
Inorganic nitrogen, e.g. nitrate, can only be transported in xylem; while organic nitrogen, e.g. amino acids, are transported in phloem.

教科書中對植物氮輸送的一句經典敘述:

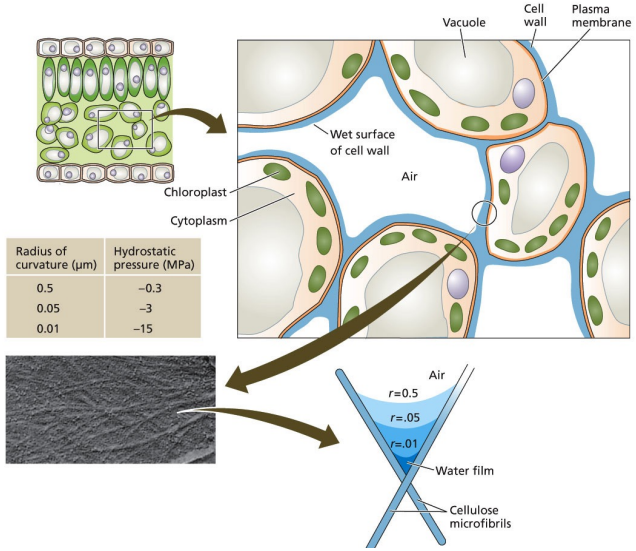
無機的氮源，例如硝酸鹽，只能靠導管輸送。
有機的氮源，例如胺基酸，才會在篩管運送。

最高的樹

- 加州 紅杉
- Redwood in California

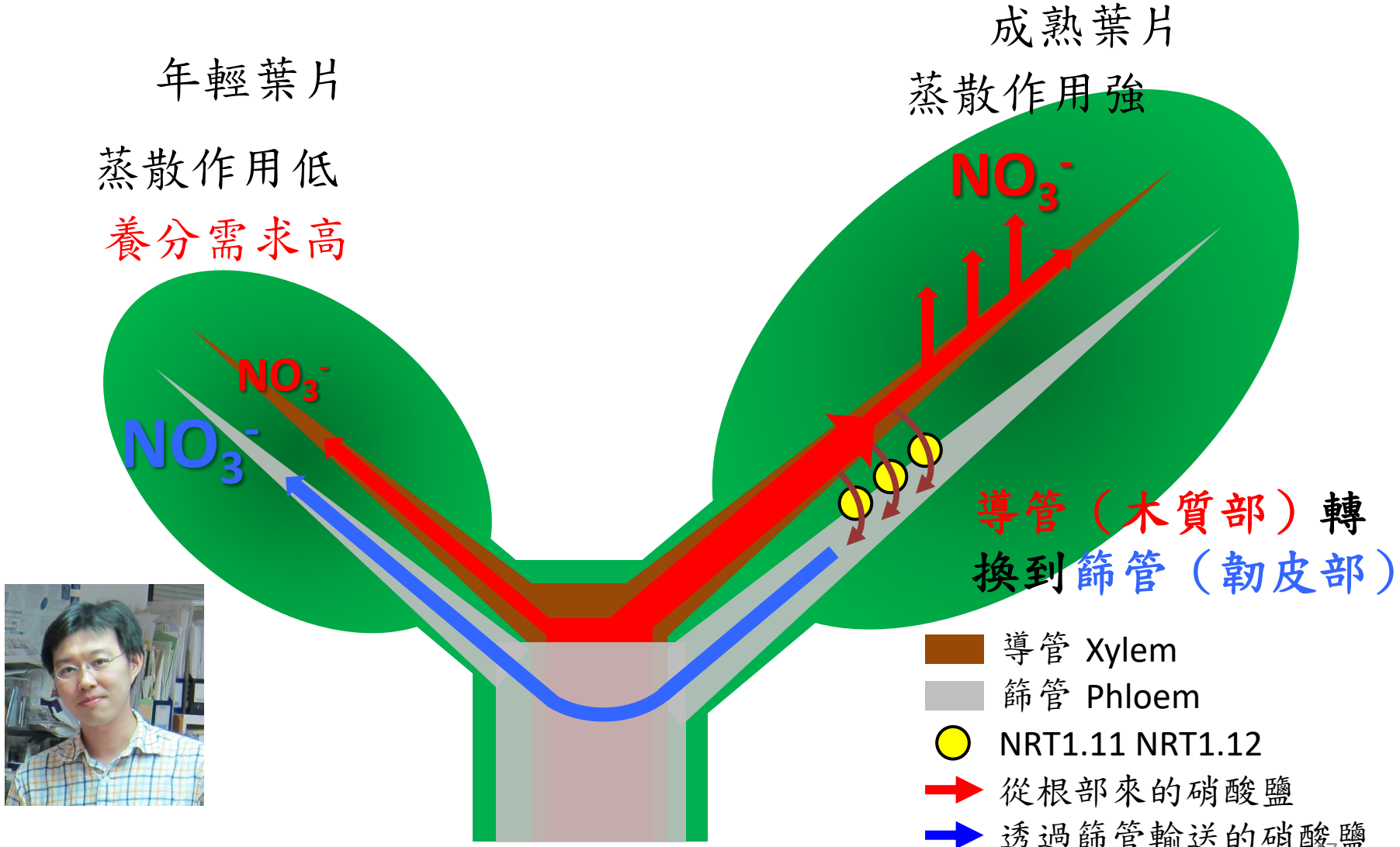


Height comparison of the Hyperion tree (iltwmt.com)



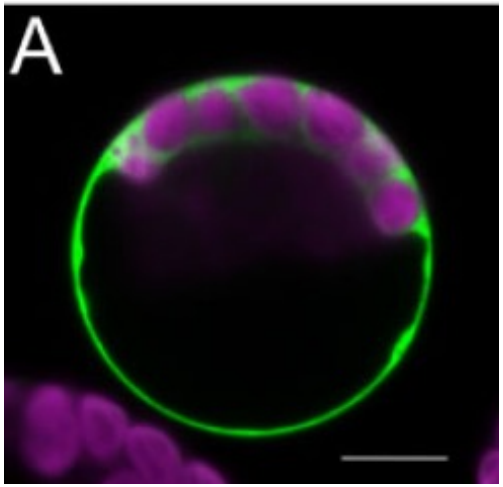
http://iliketowastemytime.com

NRT1.11 and NRT1.12 負責用換跑道的方式將硝酸鹽從成熟的大葉片送到年輕的葉片



許博凱 2013 Plant Physiol.

沒有外來的硝酸鹽供應時，植物如何餵飽嫩葉？



NO_3^-

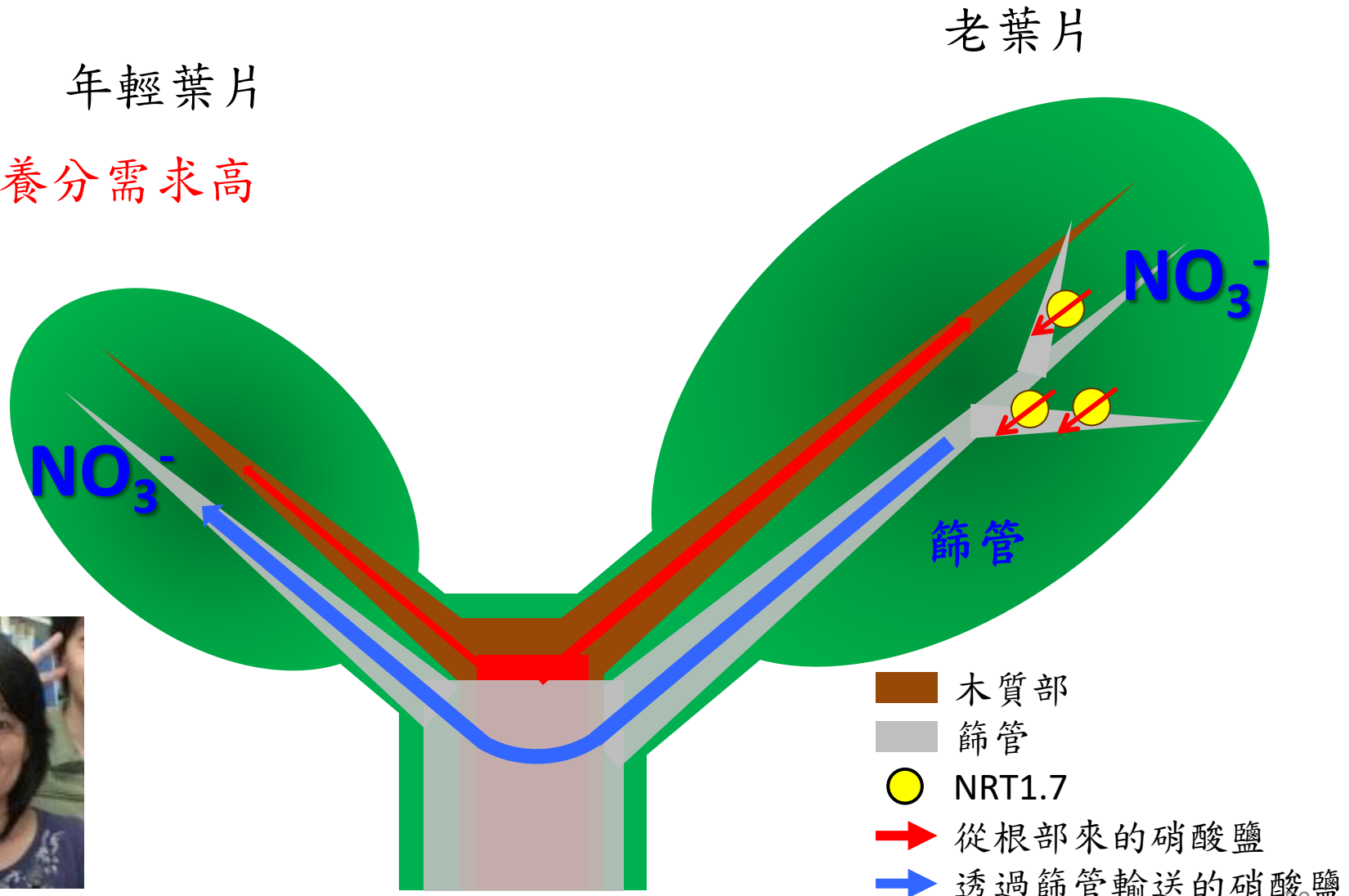
~40-70 mM

~99%

Vacuole

~5 mM

NRT1.7 負責將儲存在老葉的硝酸鹽送到年輕的葉片



范淑君 2009 Plant Cell

NRT1.11/12 and NRT1.7 play different roles in nitrate allocation

High nitrate

Low nitrate

NRT1.11/12

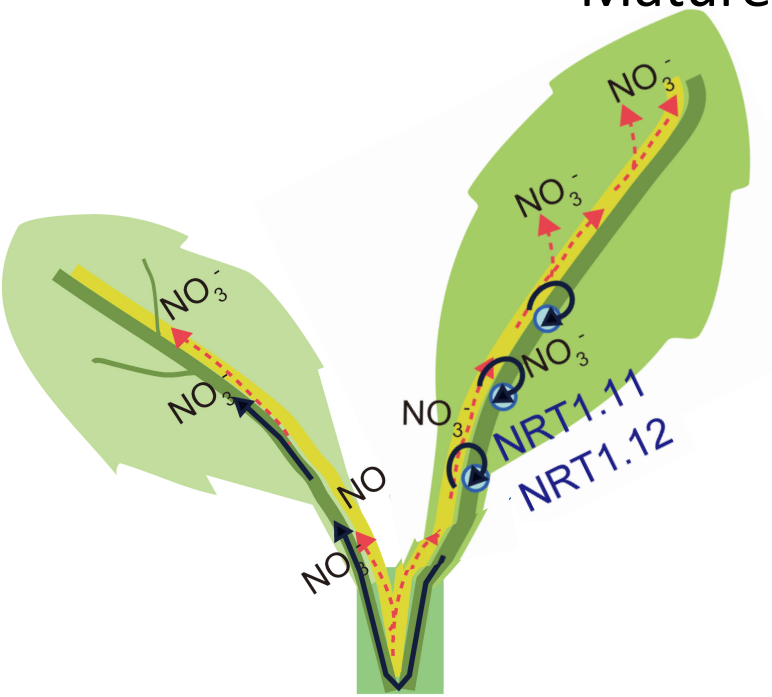
NRT1.7

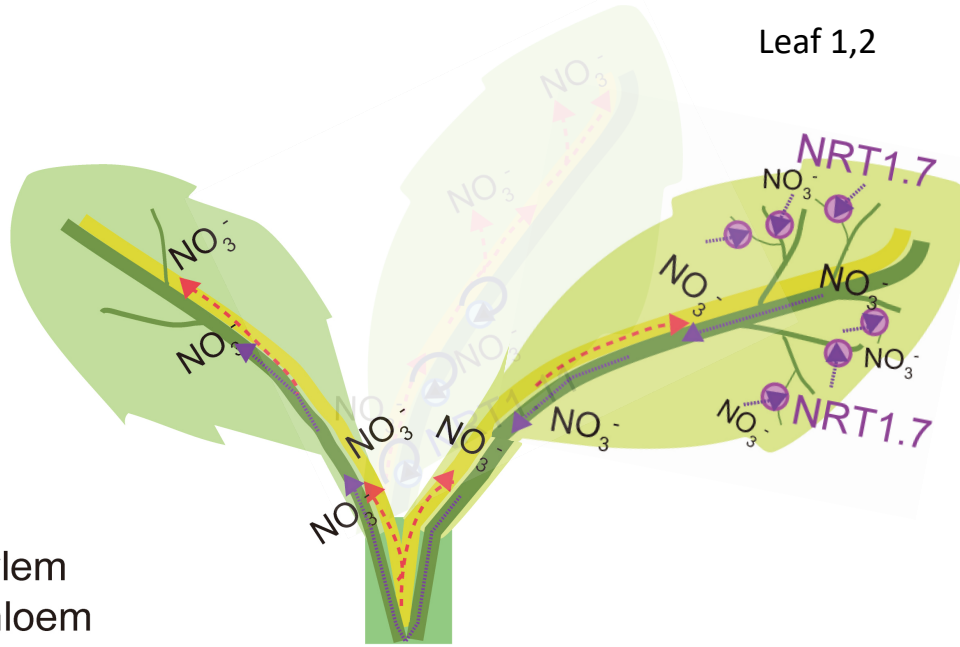
Mature leaves

Leaf 5,6

Old leaves

Leaf 1,2



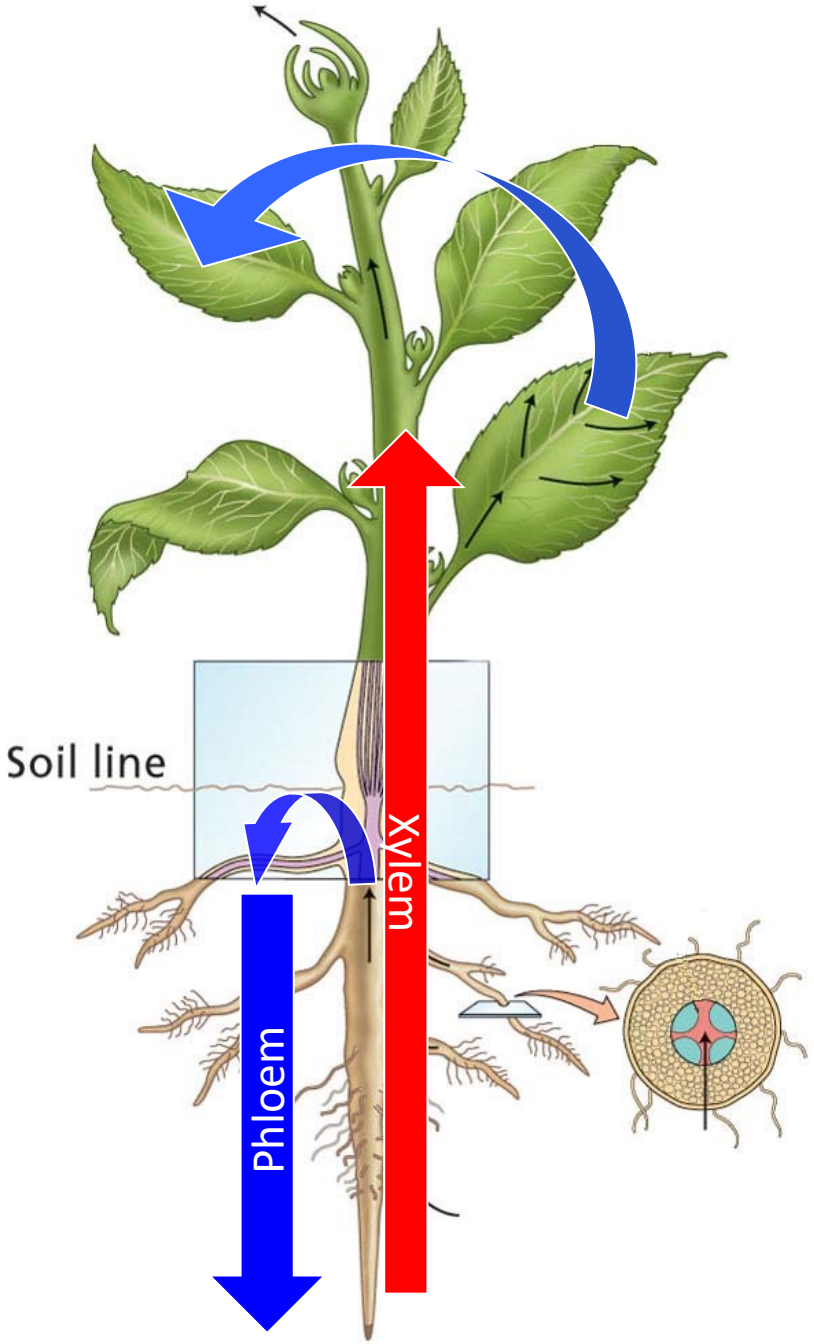


Xylem

Phloem

Xylem-borne nitrate

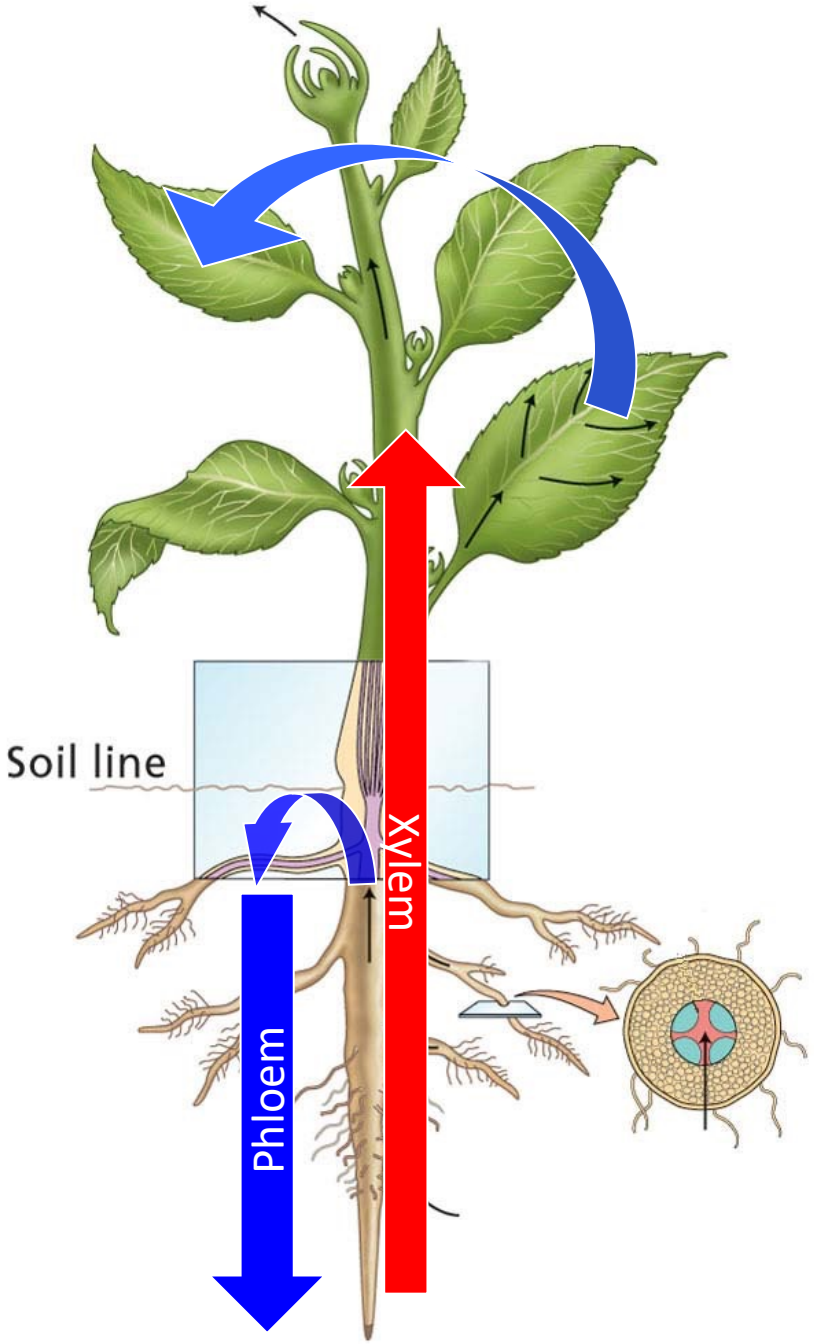
Stored nitrate



Summary 3

NRT1.9, NRT1.7, NRT1.11 and NRT1.12

- Root-to-shoot xylem transport is the primary route of long-distance nitrate transport
- Phloem nitrate transport provides a secondary route by which to modulate local nitrate redistribution.



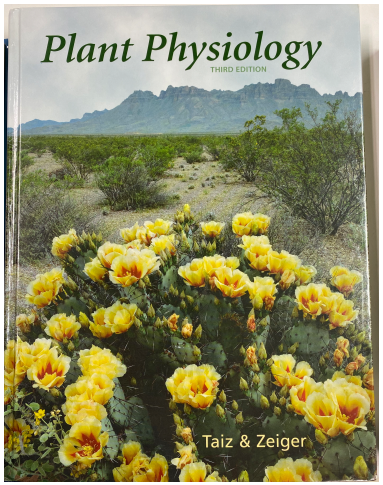
改寫教科書的理論

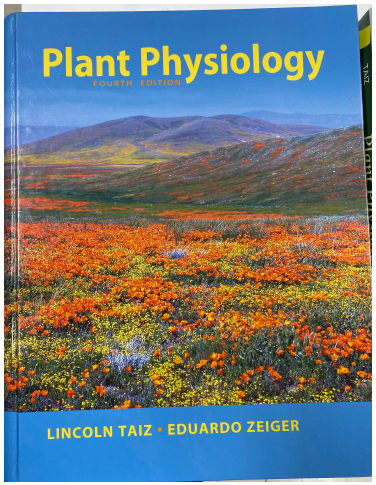
NRT1.9, NRT1.7, NRT1.11 and NRT1.12

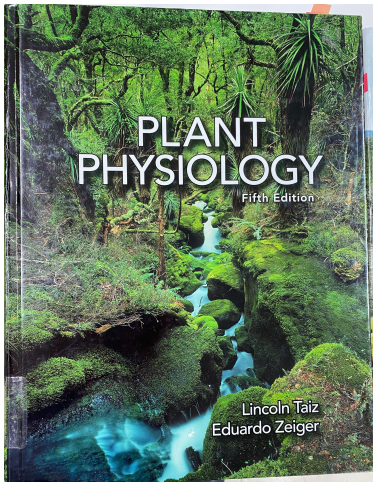
- 根往地上部輸送硝酸鹽主要是仰賴木質部Xylem
- 局部的硝酸鹽重新分佈則仰賴篩管Phloem

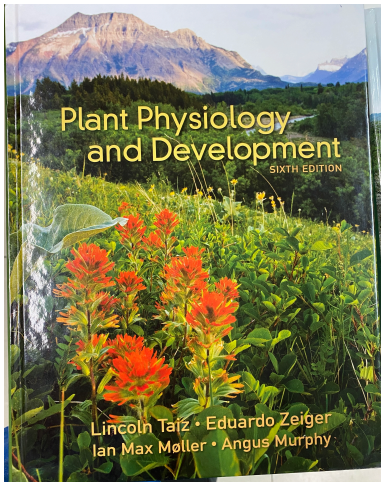
1st, 1991

2nd, 1998









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1993

CHL1: Dual Affinity
1999, 2003

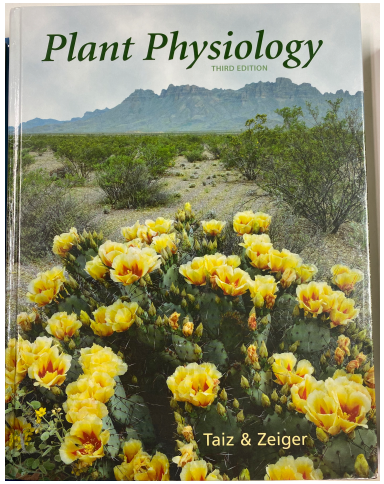
CHL1: Transceptor
2009

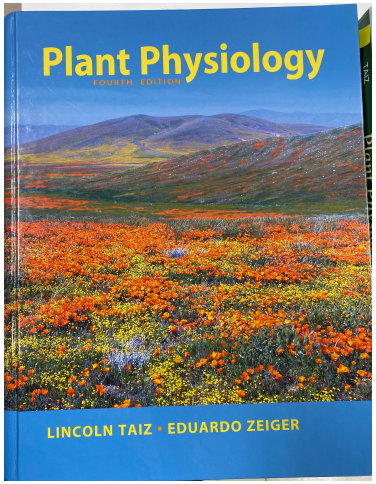
7th, 2021 or 2022

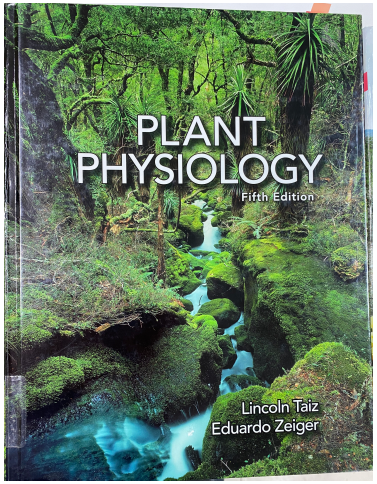
NRT1.7, 王雅筠, 2009
NRT1.9, 范淑君, 2011
NRT1.11 and NRT1.12, 許博凱, 2013

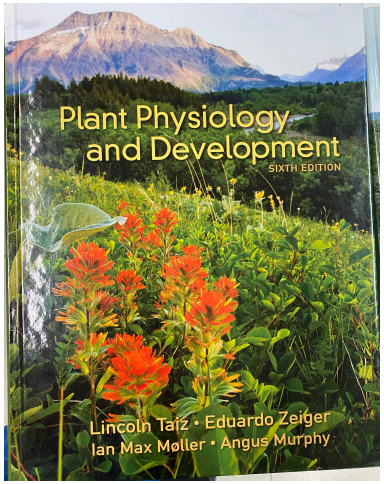
1st, 1991

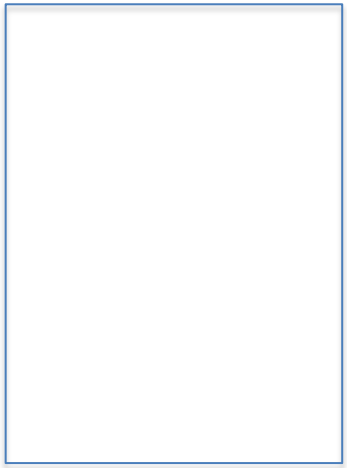
2nd, 1998











3rd, 2002

4th, 2006

5th, 2010

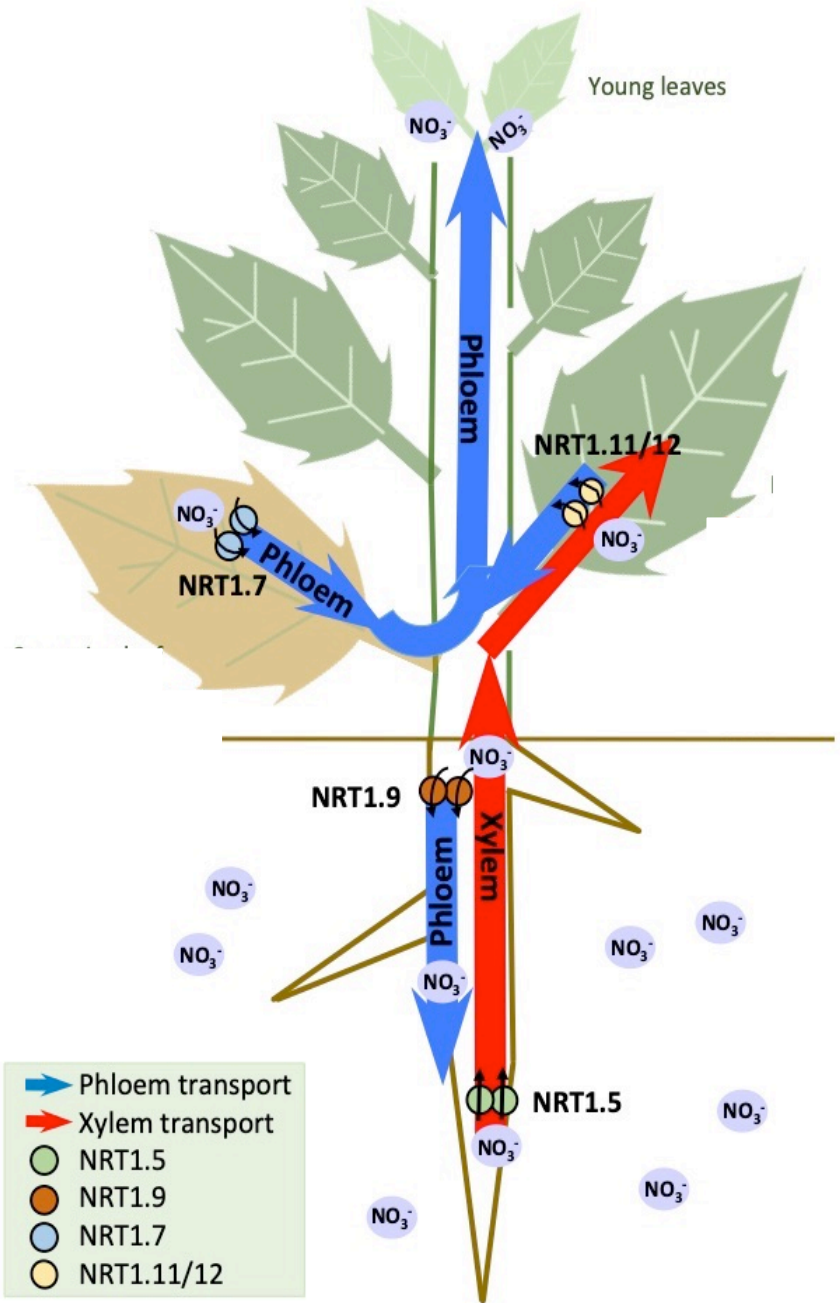
6th, 2015

7th, 2021

Taiz et al.

Phloem nitrate transport
Plant Cell, 2009
Plant Cell, 2011
Plant Physiol. 2013

NRT1.9, NRT1.7, and NRT1.11/12



Taiz et al. 7th, 2022

- Nitrate can be transported in both xylem and phloem
- Phloem nitrate transport is important for plant growth, nitrate distribution and NUE

Believe your data, more than the paper and the text book

---your data need to be believable

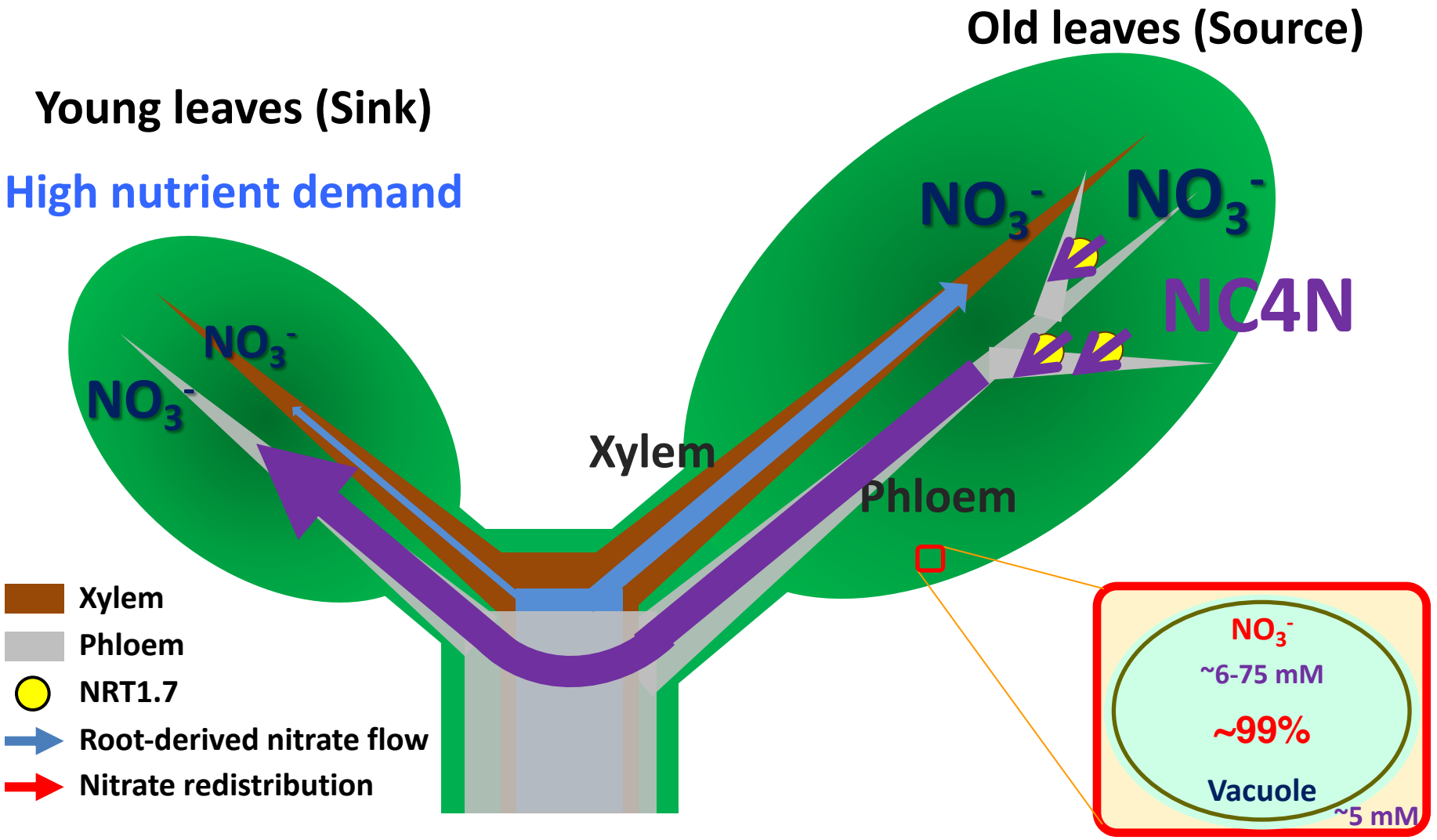
Theme 4

From basic research to application

從基礎研究到應用

浪漫的藍眼淚，其實是生態危機？

Efficient source-to-sink nitrate remobilization is

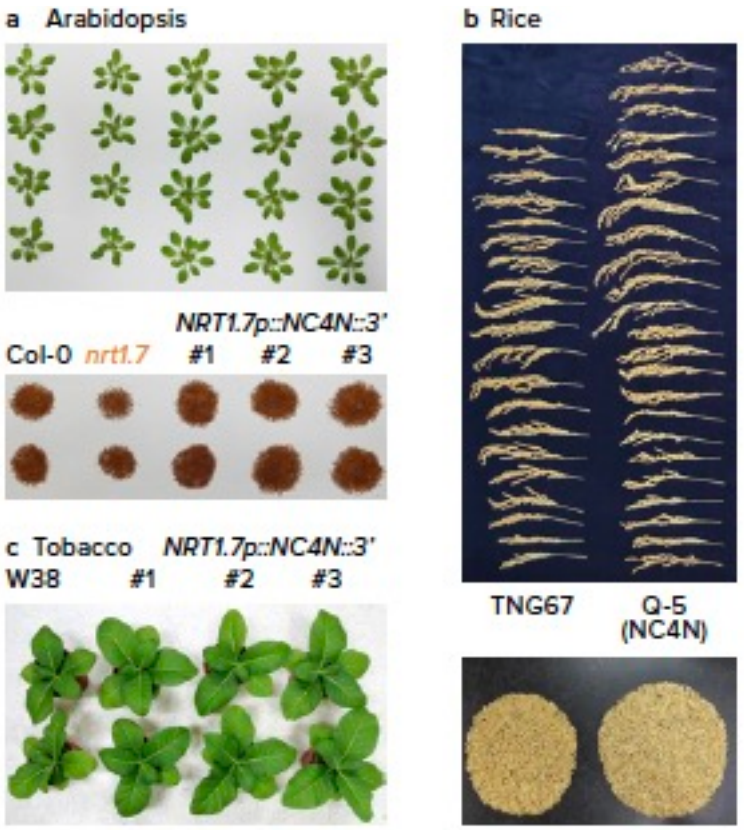


06

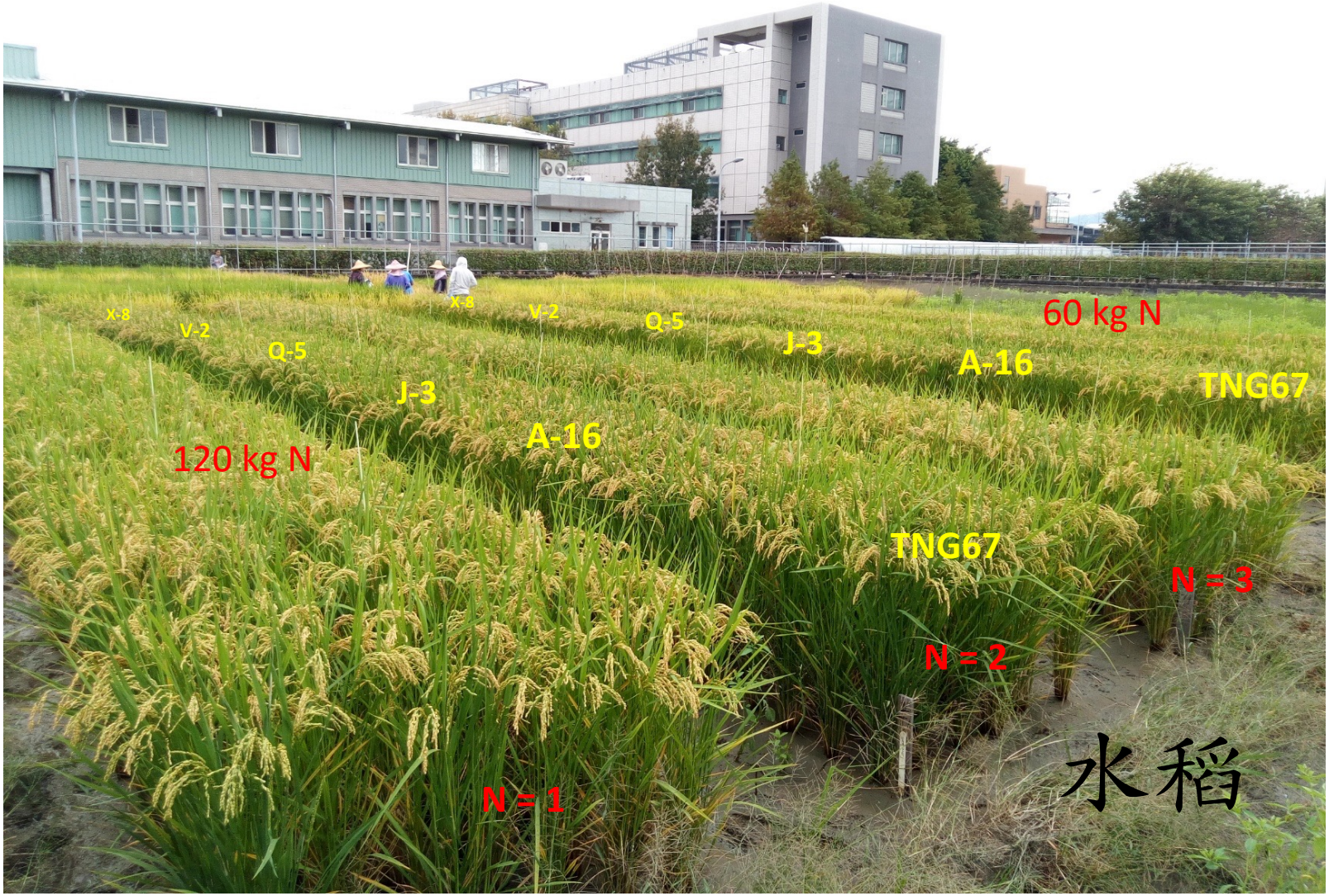
Improving nitrogen use efficiency by manipulating nitrate remobilization in plants

Kuo-En Chen[1,2], Hui-Yu Chen[1], Ching-Shan Tseng[3] and Yi-Fang Tsay[1,2]

[1]Institute of Molecular Biology, Academia Sinica
[2]Graduate Institute of Life Sciences, National Defense Medical Center
[3]Biotechnology Division, Taiwan Agricultural Research Institute



2020 Nature Plants

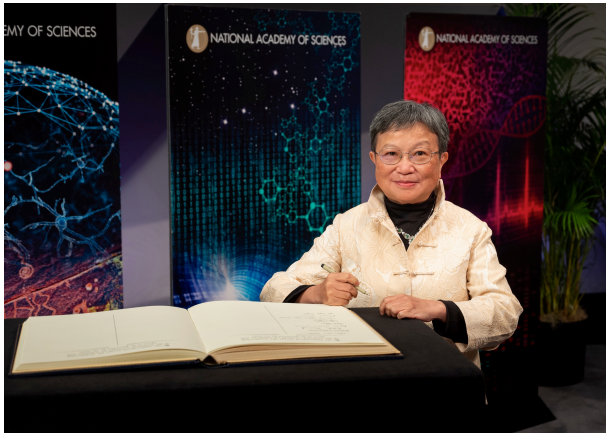

X-8
V-2
Q-5
J-3
A-16
120 kg N
X-8
V-2
Q-5
J-3
A-16
TNG67
60 kg N
TNG67
N = 3
N = 2
N = 1
水稻

Summary 4

- 提高植物體內的資源回收，可增加NUE，增加作物的產量兼顧大地的環保
- In addition to N assimilation enzyme, modulating remobilization provide a new strategy for NUE improvement.

您的得獎，應該是我來中研院後感到最激勵人心的一件事。因為這表示在中研院從助研究員開始做研究是有機會做到世界領導的研究

--林耿慧



承先啟後


Thank you