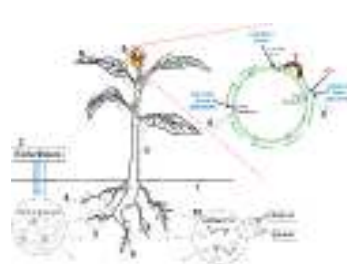
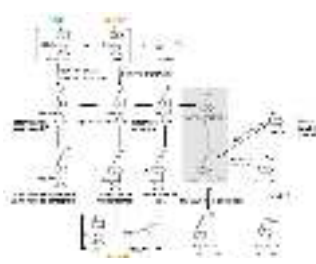


Circular systems from Water to Land: the use of Fish Manure from Aquaculture in Agriculture



Jean W. H. Yong (“John” 杨远方)



Hortikulturell produktionsfysiologi (HPF),
Institutionen för biosystem och teknologi



Swedish University of
Agricultural Sciences



“Accidental” soil scientist!

2018



Mid-West Western
Australia, Australia





Mangroves
Sustainable Growth in
SEA to FRESH-
WATER!



Eastern Thailand

Marine Fish Habitats

NORTHERN SPECIES

1 Mullet Many species of mullet feed in the soft surface waters of estuaries and mangrove creeks and are a preferred prey species of larger fish.

2 Mangrove jack *Lutjanus argentimaculatus* are widely distributed in both estuarine and reef waters along the Queensland coast and the Great Barrier Reef.

3 Red mangroves (*Rhizophora mangle*) are probably the best known mangrove plants because of their distinctive hanging aerial root systems. A common and widespread mangrove growing along coastal southern Australia from the Richmond River in New South Wales to Shark Bay in Western Australia.

4 Orange mangroves (*Avicennia marina*) are another common species in the north of Queensland. Often the most tolerant of mangroves, this species is common in mangrove communities that are subject to freshwater influences.

5 Paperbarks (*Melaleuca* species) are a common sight on the landward edge of mangrove forests, especially in the north of the State where black soil salinity is widespread, from between seaward and seaward vegetation.

6 Tiger prawns (*Penaeus* species) The black tiger prawn (*P. monodon*) lives in muddy, mangrove-lined estuaries when young and may also be found further upstream into freshwater.

Connectivity (water flow) between our varied coastal habitats is vitally important. These diverse habitats support aquatic life including fish, crabs and shellfish. Many species need to move between habitats at different times of their lives to breed and complete their life cycle. It is important to protect these habitats so that fish gain access when needed. Under the Fisheries Act 1994, all marine plants are protected, including mangroves, seagrasses, algae, saltmarsh plants and adjacent plants growing in the tidal zone.

The Department of Primary Industries has responsibility under the Fisheries Act to ensure the continuation of fish habitats and fish stocks into the future.

For more information, contact:

DPI Call Centre on 13 45 23, or visit our website at www.dpi.qld.gov.au/fishweb

Remember! No habitat = No fish!



7 Seagrasses form an important habitat in providing food and shelter for many species of fish, crabs and prawns, and in trapping sediments and stabilising the sea bed. *Posidonia* is widely found in a common species of northern Queensland estuaries and bays.

8 Barramundi (*Lateolabrax*) habitat connectivity is critical to barramundi as they migrate from rivers to the coast to breed and spend their early life in sheltered creeks, lagoons, mangroves and mangrove swamps.

9 Mud crabs (*Decapoda*) are found along the length of the Queensland coast, inhabiting estuaries, tidal flats and mangrove fringe areas.

10 Twiscope mud whelks (*Strophomena*) grow to 10cm. These large conical shells are found in northern Australian estuarine mudflats.

11 Sedges are common in the vegetation fringe bordering the brackish reaches of tidal streams.

Vital community assets

Managing our fish habitats



Department of
Primary Industries and Fisheries
Queensland Government

Aquaculture and **Mangroves** can **Co-EXIST**

Surabaya, Java, Indonesia



Mangroves growing in freshwater



Christmas island, Australia



Aquaculture and Mangroves can Co-EXIST



Yogyakarta, Java, Indonesia

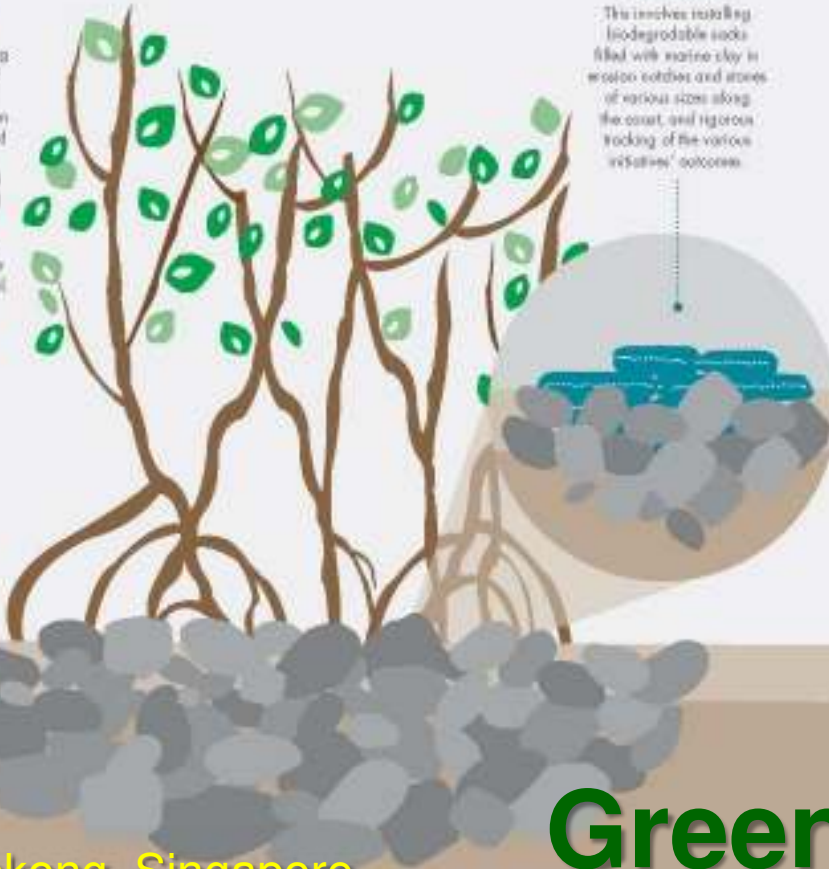


RESTORING PULAU TEKONG'S MANGROVES

In the past, scouring – where mud is washed away by water swirling around tree roots – was observed, causing shoreline erosion. Higher sea levels are also expected to further degrade the mangrove forests in Pulau Tekong. To counter this, we have applied both "hard" and "soft" engineering solutions.

SOFT APPROACH

This involves planting multiple species of native mangrove saplings, grown from propagules collected from all over Singapore, instead of the conventional single-species planting, thereby restoring our native mangrove gene pool.



The northeastern coastline of Pulau Tekong accounts for

90
HECTARES
of pristine mangrove forest.

HARD APPROACH

This involves installing biodegradable sacks filled with marine clay in erosion patches and stones of various sizes along the coast, and rigorous tracking of the various initiatives' outcomes.



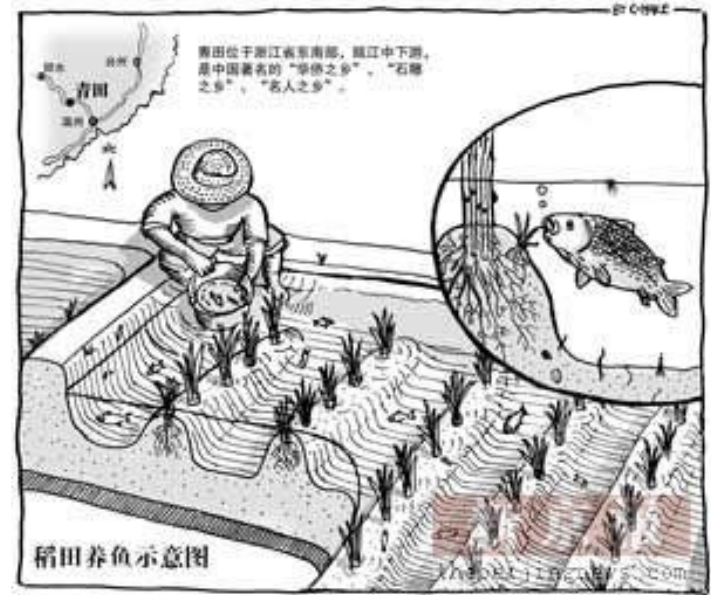
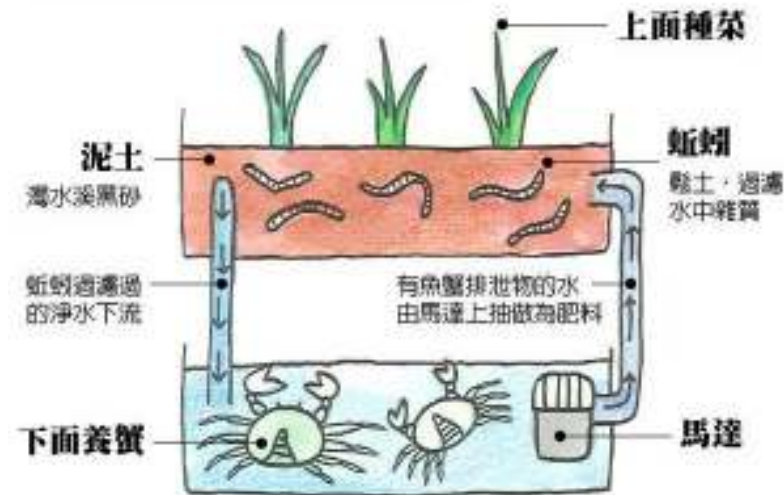
Replanting Mangroves

**Biodegradable
Bags**

Biodegradable Rings



Rice – Animals Co-cultivation



Prof Xin Chen, Zhejiang University





Rice – Fish Co-cultivation



Xie et al (2011) PNAS



Are there natural **Biostimulants** in animal waste?



Cyprinus carpio



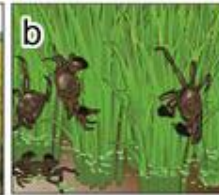
A Rice-carp system (Zhejiang)



Eriocheir sinensis



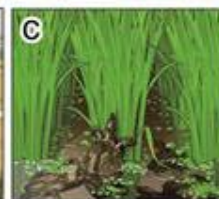
B Rice-crab system (Liaoning)



Pelodiscus sinensis



C Rice-turtle system (Zhejiang)



Procambarus clarkii



D Rice-crayfish system (Anhui)



Misgurnus anguillicaudatus



E Rice-loach system (Chongqing)





**Earthworms
(vermi-compost)**

Natural Soil Fertility

Darwin on Earthworms: The
Formation of Vegetable
Mould Through the Action
of Worms With
Observations on Their
Habits by Charles Darwin

Darwin, Charles

Note: This is not the actual book cover.

Darwin ON Earthworms

THE FORMATION
OF VEGETABLE MOULD
*Through the Action of Worms
With Observations on
their Habits* by
Charles Darwin

with a foreword by
James P. Martin Ph.D.
Staff Scientist, 1961, University of California

Charles Darwin

Earthworms (vermi-compost)



Perionyx excavatus and *Eisenia foetida*
at 65:35 ratio

Large-scale Biostimulants' usage & Soil Re- constitution



Past Successful Track Record

Lampung, Sumatra, Indonesia



PT Great Giant Pineapple

Types of Fertilizers

- Chemical (NPK)
- Traditional (manure)
- Organic/**Green**



Traditional, Organic/**Green** **Fertilizers**

- Composts, Vermi-compost (earthworms)
- Liquid/pellet microbial applications
- Humo-organic

Biostimulants

Plant biostimulants are diverse substances and microorganisms used to enhance **plant growth**.

Biostimulants

Eight categories (Calvo et al. 2014)

- Microbial inoculants
- Humic acids (including Fulvic acids)
- Protein hydrolysates & amino acids (including **MicroProteins**)
- Seaweed extracts
- Complex organic materials (including **phytohormones**)
- Beneficial chemical elements & Inorganic salts (including phosphite)
- Chitin and chitosan derivatives
- Anti-transpirants

Plant Soil (2014) 557:3–41
DOI 10.1007/s11104-014-4212-6

MARSCHNER REVIEW

Agricultural uses of plant biostimulants

Parrita Calvo · Louise Nelson · Joseph W. Klugger

Received: 20 December 2013 / Accepted: 25 April 2014 / Published online: 6 May 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract

Background Plant biostimulants are diverse substances and microorganisms used to enhance plant growth. The global market for biostimulants is projected to increase 12.8 % per year and reach over \$2,100 million by 2015. Despite the growing use of biostimulants in agriculture, many in the scientific community consider biostimulants to be lacking peer-reviewed scientific evaluation.

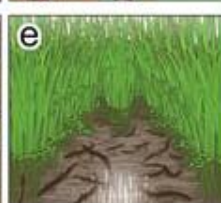
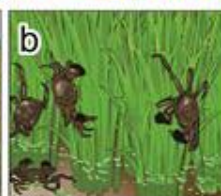
Aims: This article describes the emerging definitions of biostimulants and reviews the literature on the strategies

Keywords Microbial inoculants · Humic acids · Fulvic acids · Protein hydrolysates · Amino acids · Seaweed extracts · Biostimulants

Introduction

Plant biostimulants, or agrobiostimulants, include diverse substances and microorganisms that enhance plant growth. The global market for biostimulants has been projected to reach \$2,241 million by 2015 and

Are there natural **Biostimulants** in animal waste?



Dr Markus Langeland
(SLU Uppsala)



SCIENTIFIC REPORTS

Can the co-cultivation of rice and fish help sustain rice production?



Vermicompost tea – a leachate



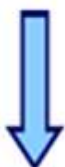
- This is the first mass spectrometric and therefore unequivocal evidence for the presence of CKs in VT: namely **trans-Zeatin (tZ)**, **N⁶-Isopentenyladenine (iP)** and **N⁶-Isopentenyladenosine (iPR)**, and were determined to be present at 0.06, 3.33, & 0.02 pmol mL⁻¹, respectively.
- The successful detection and quantitation of CKs in VT provided direct evidence to explain the growth efficacy of applying VT to enhance plant growth and development.
- We postulated that **iP is a good reflection of the microbial origin for CKs present in the VT due to its importance in CK biosynthesis pathways** and high abundance provided by microorganisms.



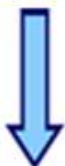
Zhang et al. 2014, Biol Fert Soils



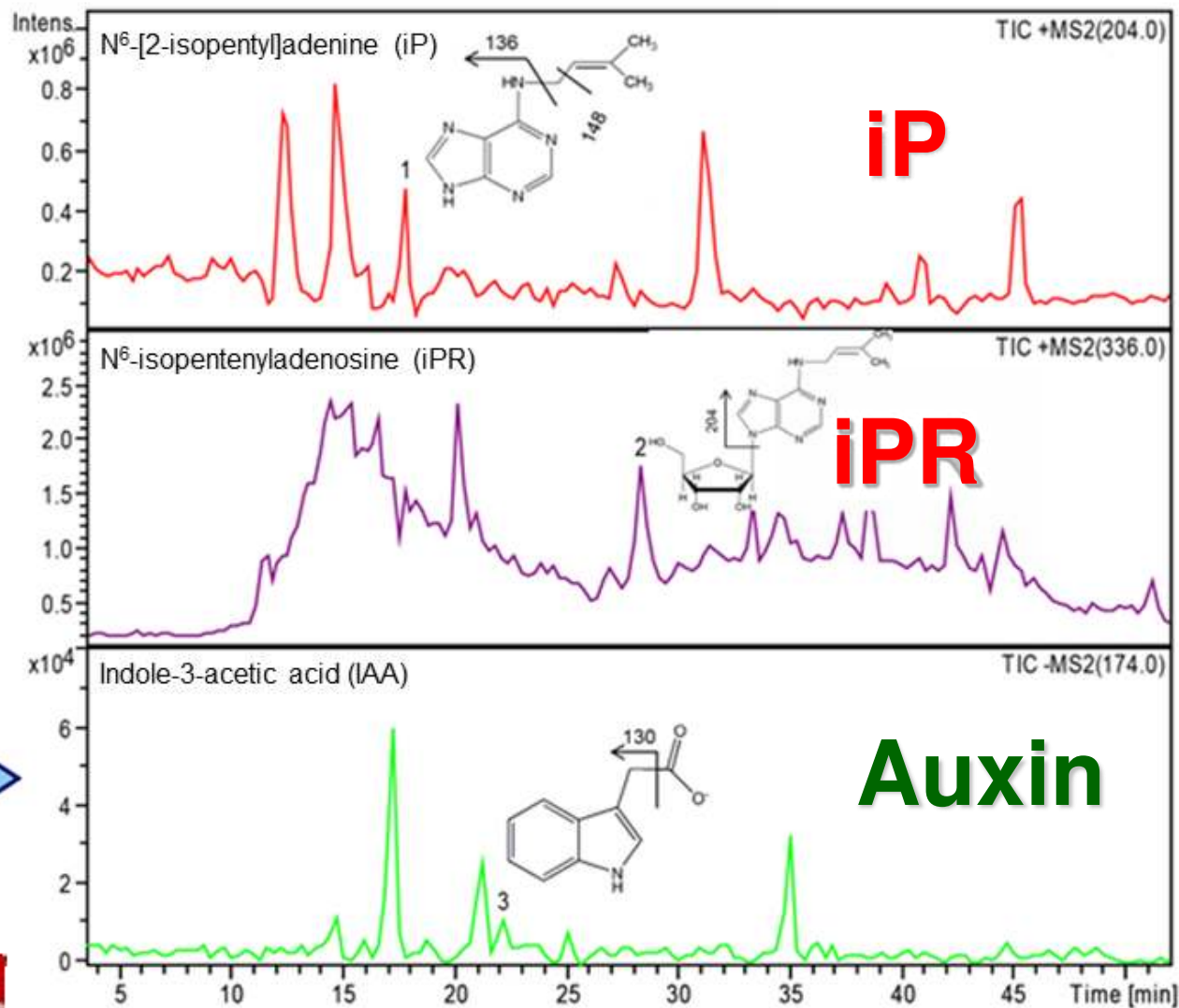
Vermicomposts



1.UAE
2.SPE



LC-MS
analysis



Vermicompost tea – Other Research Groups

Plant Growth Regul (2016) 19:482–492
DOI 10.1007/s10255-014-0401-0

ORIGINAL PAPER

Evidence of phytohormones and phenolic acids variability in garden-waste-derived vermicompost leachate, a well-known plant growth stimulant

Adagomi O. Arima · Wendy A. Sirek · Manoj G. Kulkarni · Eamonn Turkouski · Veronika Turečková · Jiri Gruz · Michaela Subrtová · Aleš Pěnčík · Ondřej Novák · Karel Doháňal · Miroslav Strnad · Johannes Van Staden

Received: 7 October 2014 / Accepted: 10 December 2014 / Published online: 18 December 2014
© Springer Science+Business Media Dordrecht 2014

Abstract Cytokins, auxins, abscisic acid, gibberellins (GA) and brassinosteroids (BRs) as well as the phenolic acid content in three batches of vermicompost leachate (VCL) were quantified using ultra-high performance liquid chromatography tandem mass spectrometry. *N*⁶-isopentenyladenine formed the major (60–94 %) proportion of the CK content. *o*-Hidra dihydrozeatin had the lowest (<0.02 %) concentration. Indole-3-acetic acid ranged from approximately 0.55–0.77 $\mu\text{mol/L}$. A total of 18 GAs including bioactive forms and inactive end products were observed in the VCL samples. Caffeoylquinic acid had the highest (3,900–9,200 $\mu\text{g/mL}$) concentration while brassinolide was the lowest (1–5 ng/mL) abundant BRs found. Phenolic acids quantified were gallicacetic acid (3–3.8 $\mu\text{g/mL}$), *p*-hydroxybenzoic acid (2.5–2.8 $\mu\text{g/mL}$), *p*-coumaric acid (1–1.7 $\mu\text{g/mL}$) and ferulic acid (0–1 $\mu\text{g/mL}$). These results provide an indication of the rich diversity in natural PGRs and phytochemicals in VCL which may indirectly contribute to the numerous favorable physiological responses elicited by VCL application to plants.

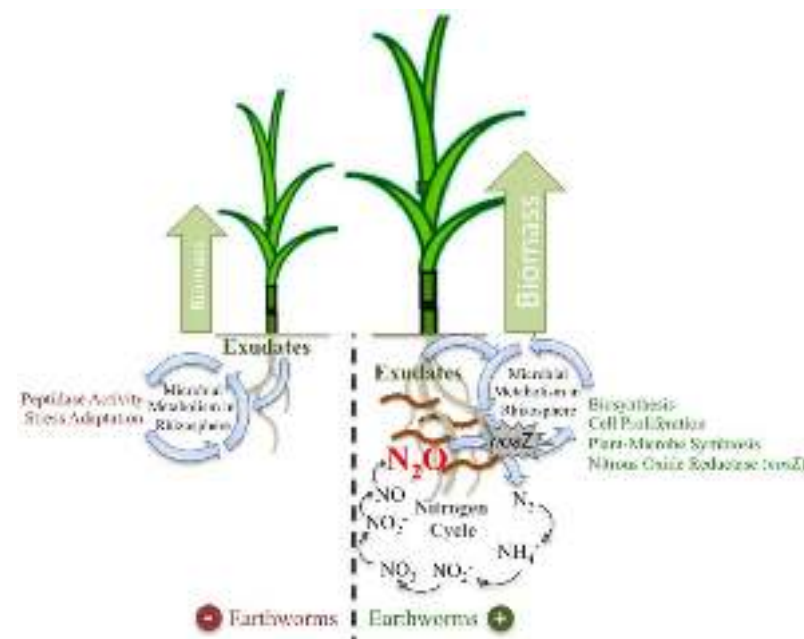
Keywords Abscise acid · Biostimulant · Brassinosteroids · Cytokines · Gibberellins · Phenolics

Abbreviations

ABA	Abscise acid
BRs	Brassinosteroids
CKs	Cytokins
<i>o</i> -H	<i>o</i> -Zeatin
<i>o</i> -ZHG	<i>o</i> -Zeatin 9-glucoside
<i>o</i> -ZOG	<i>o</i> -Zeatin- <i>O</i> -glucoside
<i>o</i> -Z	<i>o</i> -Zeatin chloride
<i>o</i> -ZMIP	<i>o</i> -Zeatin riboside-5'-monophosphate
<i>o</i> -ZROG	<i>o</i> -Zeatin- <i>O</i> -glucoside chloride
DH2	Dihydrozeatin
DH2OG	Dihydrozeatin-9-glucoside
DH2OG	Dihydrozeatin- <i>O</i> -glucoside
DH2Z	Dihydrozeatin chloride
DH2MIP	Dihydrozeatin riboside-5'-monophosphate
DH2ROG	Dihydrozeatin- <i>O</i> -glucoside chloride
GA	Gibberellins
IAA	Indole-3-acetic acid
LC/MS	Immunoaffinity chromatography

In addition
to **Auxins**
and
Cytokinins

+ **Gibberellins**
+ **Brassinosteroids**



Kinetin

Regular Article

Identification of 6-Furfuryladenine (Kinetin) in Human Urine

Jan Barciszewski^{a, *}, Michał Mielcarek^a, Maciej Stobielecki^a, Gunhild Siboska^b, Brian F.C. Clark^b

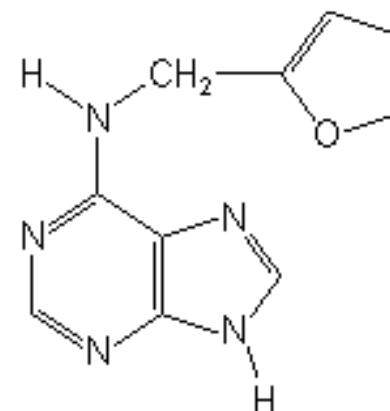
[Show more](#)

<https://doi.org/10.1006/bbrc.2000.3928>

[Get rights and content](#)

Abstract

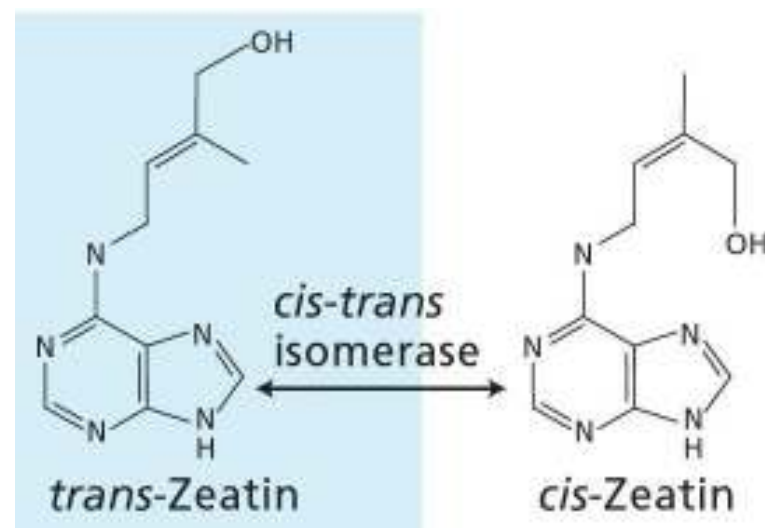
In contrast to the current view of kinetin (K, *N*⁶-furfuryladenine) as an unnatural and synthetic cytokinin, recently it has been identified in plant DNA and plant extract. Here we describe identification of K in human urine using



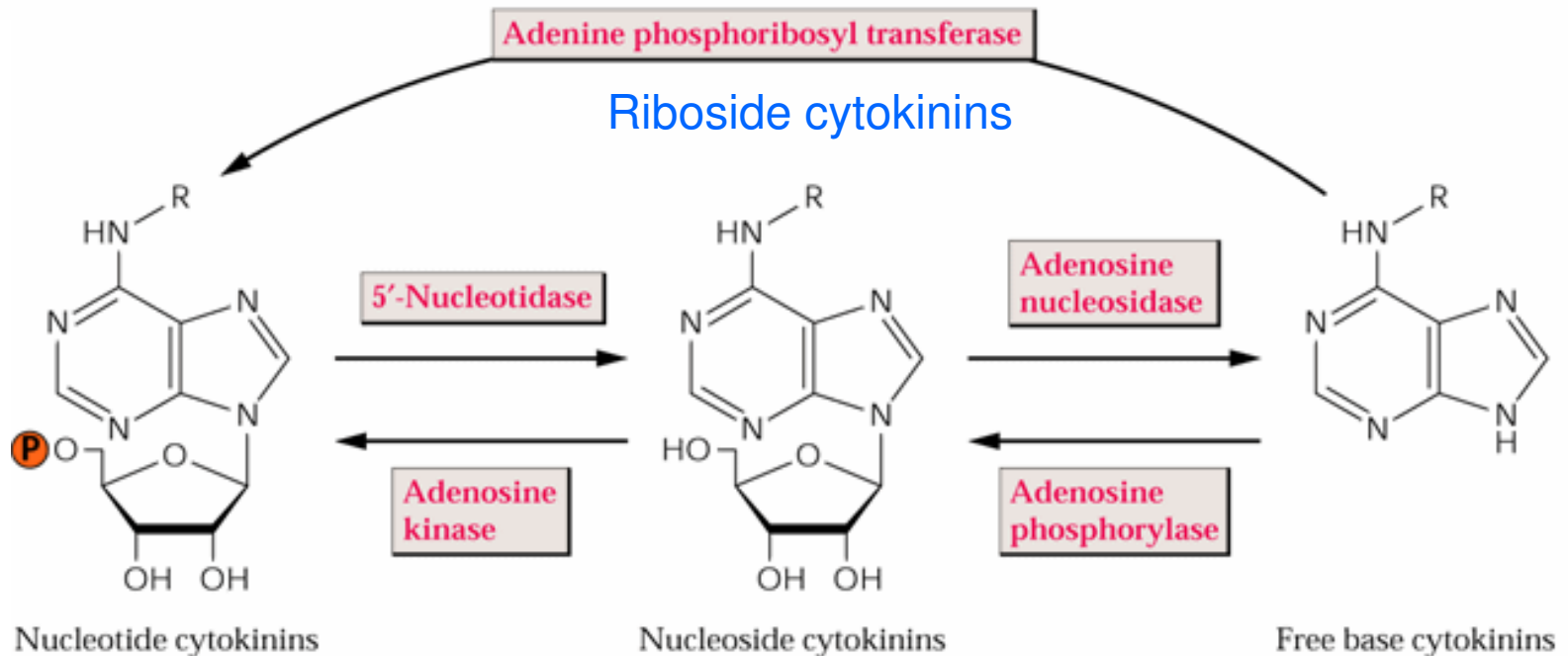
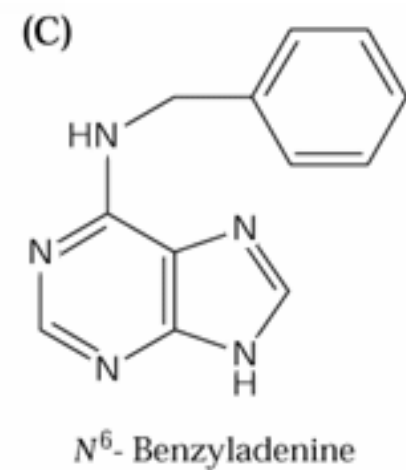
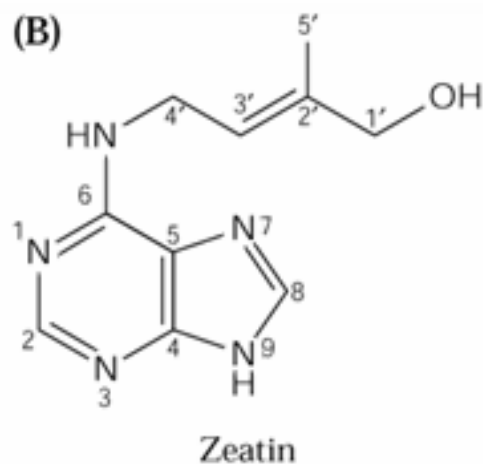
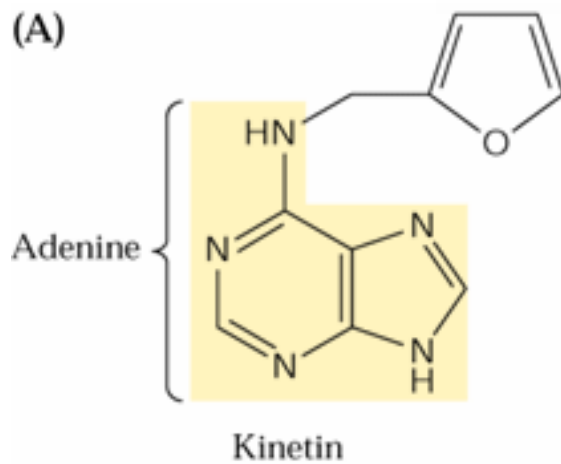
?



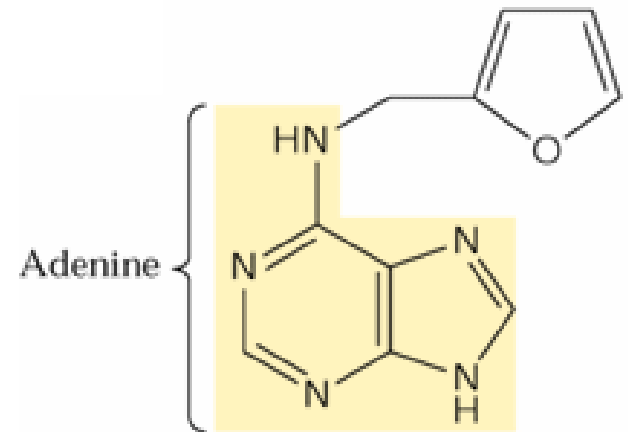
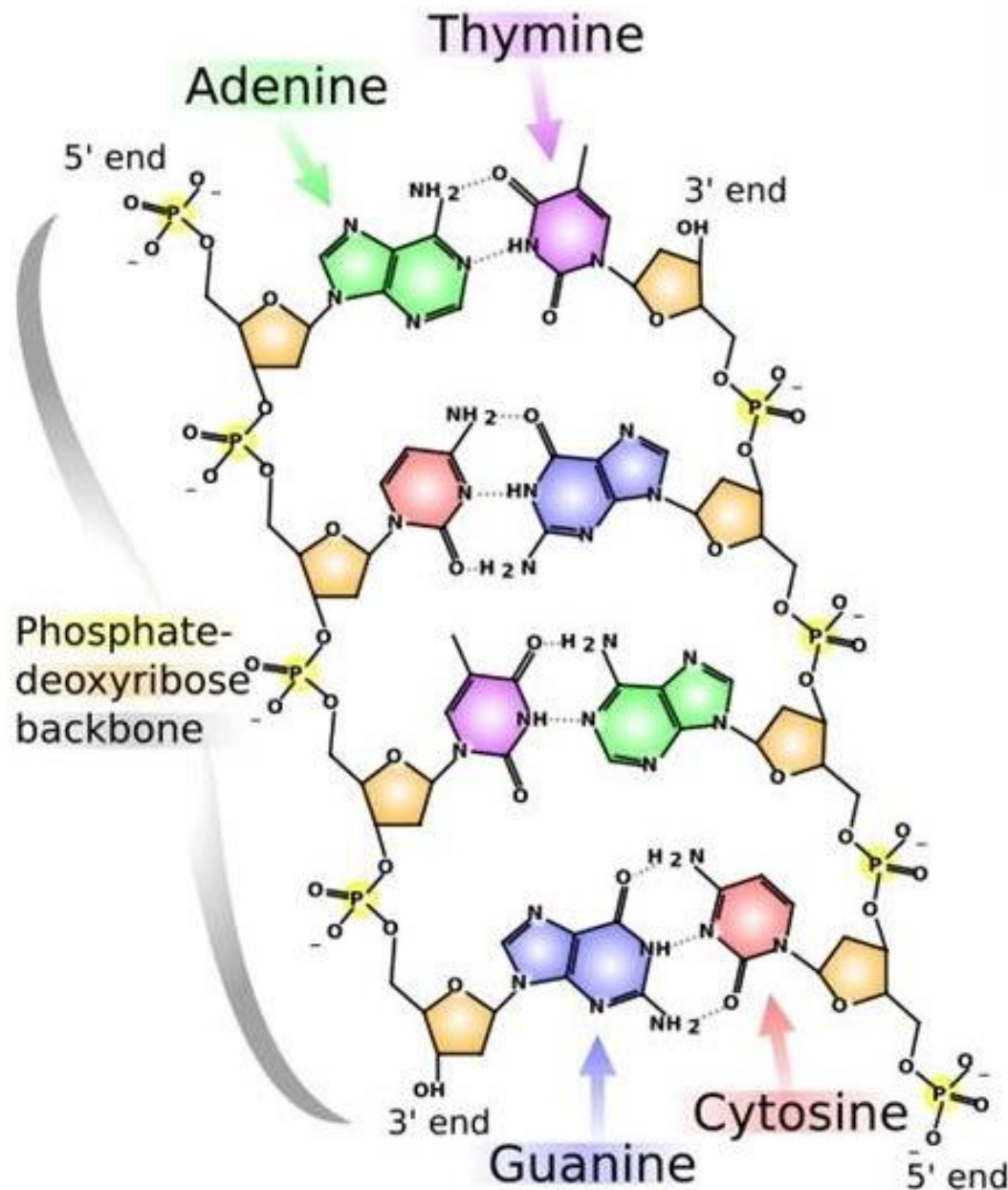
?



Cytokinin types

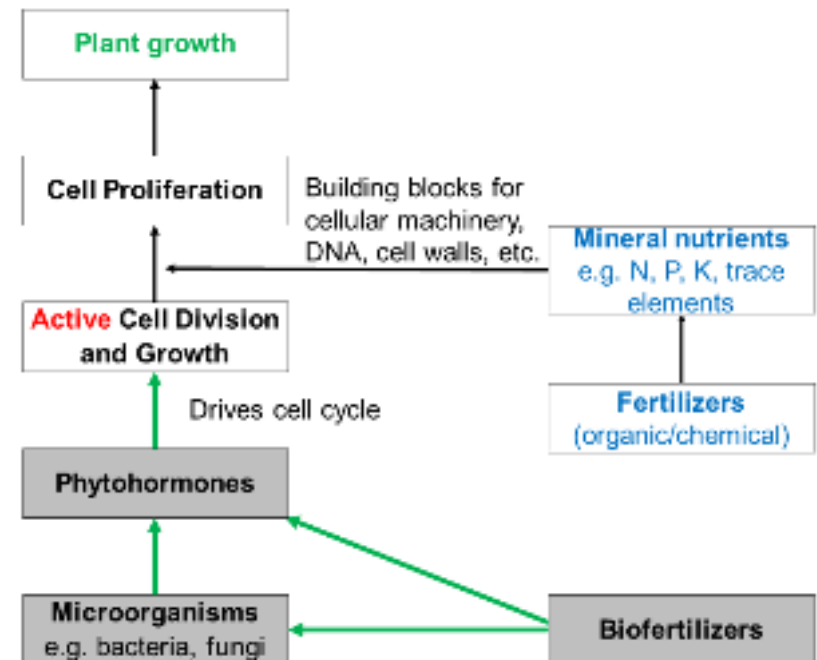
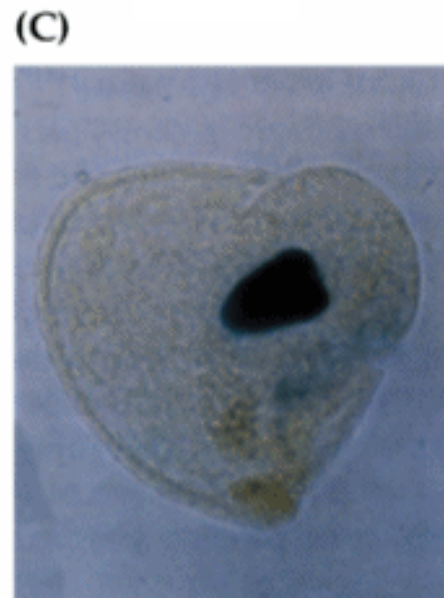
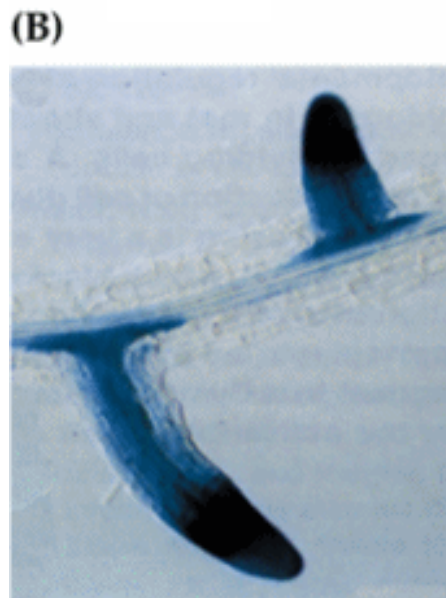
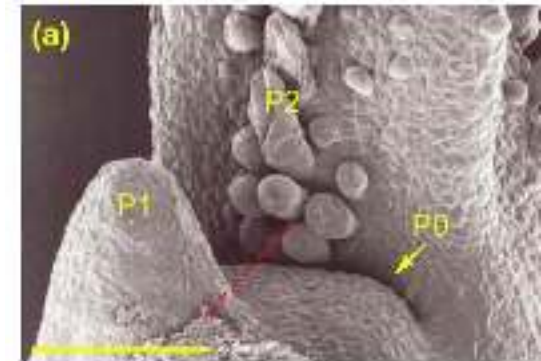
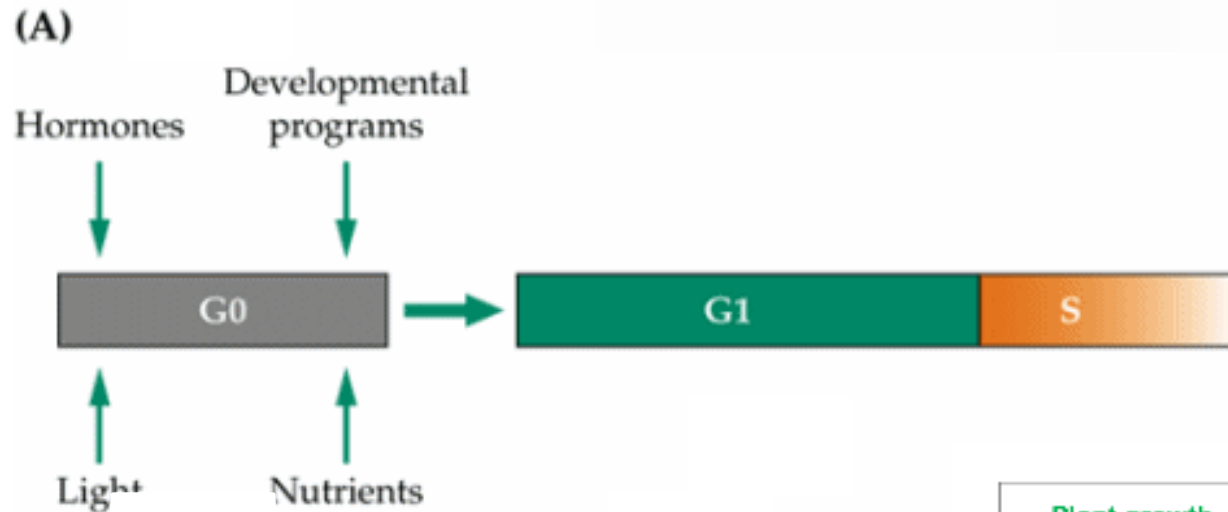


Components of the DNA



Kinetin

How Phytohormones drive the Cell Cycle?



Plants can use protein as a nitrogen source without assistance from other organisms

Chanyarat Paungfoo-Lonhienne*, Thierry G. A. Lonhienne†, Doris Rentsch†, Nicole Robinson*, Michael Christie†, Richard I. Webb§, Harshi K. Gamage*, Bernard J. Carroll†, Peer M. Schenk*, and Susanne Schmidt*¶

*School of Integrative Biology, TARC Centre of Excellence for Integrative Legume Research, School of Molecular and Microbial Sciences, and School of Land Crop and Food Sciences, and †Centre for Microscopy and Microanalysis, University of Queensland, Queensland 4072, Australia; and ‡Institute of Plant Sciences, University of Bern, 3013 Bern, Switzerland

Edited by Peter Vitousek, Stanford University, Stanford, CA, and approved January 25, 2008 (received for review December 21, 2007)

Nitrogen is quantitatively the most important nutrient that plants acquire from the soil. It is well established that plant roots take up nitrogen compounds of low molecular mass, including ammonium, nitrate, and amino acids. However, in the soil of natural ecosystems, nitrogen occurs predominantly as proteins. This complex organic form of nitrogen is considered to be not directly available to plants. We examined the long-held view that plants depend on specialized symbioses with fungi (mycorrhizas) to access soil protein and studied the woody heathland plant *Hakea actites* and the herbaceous model plant *Arabidopsis thaliana*, which do not form mycorrhizas. We show that both species can use protein as a nitrogen source for growth without assistance from other organisms. We identified two mechanisms by which roots access protein. Roots exude proteolytic enzymes that digest protein at the root surface and possibly in the apoplast of the root cortex. Intact protein also was taken up into root cells most likely via endocytosis. These findings change our view of the spectrum of nitrogen sources that plants can access and challenge the current paradigm that plants rely on microbes and soil fauna for the breakdown of organic

(13), (ii) most other heathland plants have mycorrhizal symbioses and/or form symbioses with N_2 -fixing microbes (13), and (iii) *Hakea* forms cluster roots that have a role in the acquisition of organic nitrogen (14). We further hypothesized that *Arabidopsis thaliana* is unable to use protein as a nitrogen source because it does not form cluster roots and grows in ruderal habitats that typically contain inorganic nitrogen. With the aid of fluorescent proteins, we show that both plant species, in the absence of microbes, can use protein as a nitrogen source.

Results

Axenically Cultivated *H. actites* and *A. thaliana* Use Externally Supplied Protein as a Nitrogen Source for Growth. Grown with protein as the sole nitrogen source, *Hakea* seedlings produced significantly more root biomass and had a greater nitrogen content in roots than plants grown without nitrogen (Fig. 1*A* and *B*). Shoot biomass and shoot nitrogen content, as well as total plant biomass and nitrogen content, were similar in *Hakea* grown without nitrogen or with protein, and best shoot and root growth

(Fig. 1*C* and *D*). *Arabidopsis* grown with a low amount of inorganic nitrogen (20 mg NH_4NO_3 per ml of growth medium) produced more above-ground and total plant nitrogen than plants grown with 5 mg BSA per ml growth medium (Fig. 1*E* and *F*). *Arabidopsis* grown with a mixture of protein and a low amount of inorganic nitrogen (5.4 mg BSA per ml and 0.04 mg NH_4NO_3 per ml)

nitrogen
soil protein

New Ideas, Original Thinking?

Soil organic matter contains nitrogen predominantly as protein, which is considered a nitrogen source exclusively for microbes and animals (1, 2). Despite the importance of protein in soils, little research has been carried out to elucidate the use of complex organic nitrogen as a nitrogen source for plants.



PNAS 2008



Ascophyllum



Kelp



Gunapaseelam

(Fermented Fish waste)

Kunapajala

(Fermented animal waste, bones. etc)



Water

Urea

Gunapaseelam



Phytohormones



NTU Dr Ge Liya

Coconut water



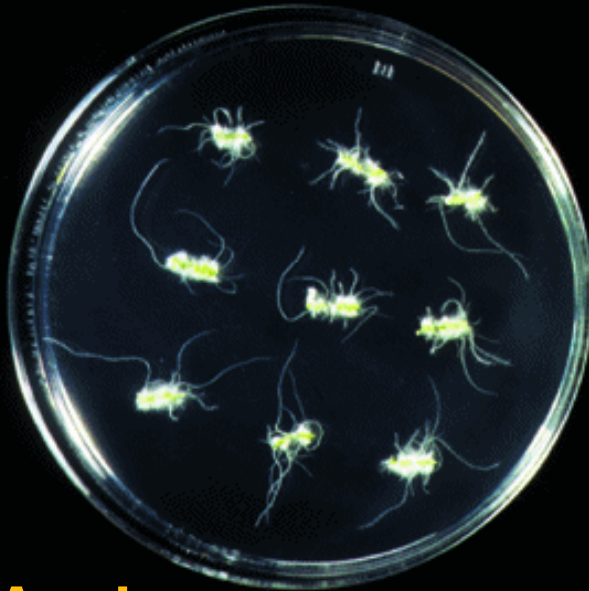
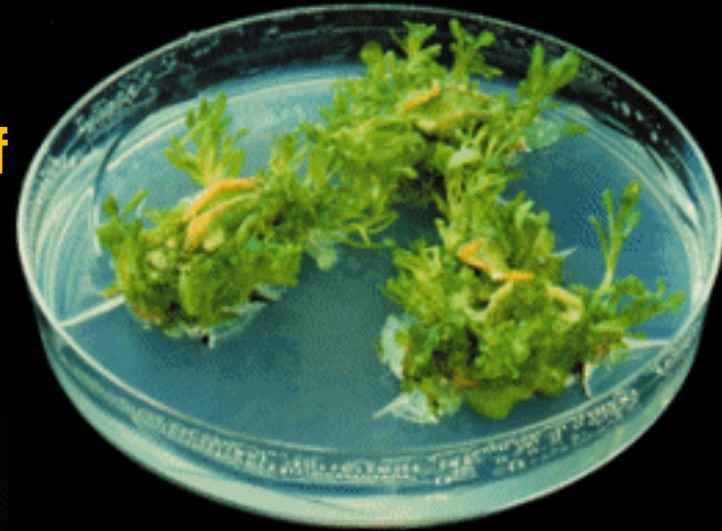
Yong et al. 2009, Molecules

Insights from Tissue Culture

Analogy – Reductionist approach?

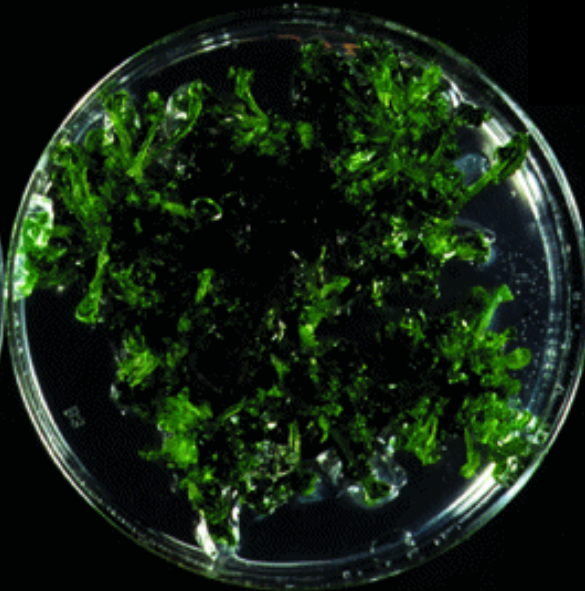
Plant Tissue Culture

**Mineral Nutrients +
Full complement of
phytohormones**



Auxins

IBA, $0.5 \mu\text{g ml}^{-1}$



IBA, $0.5 \mu\text{g ml}^{-1}$
Zeatin, $2.0 \mu\text{g ml}^{-1}$

**Auxins +
Cytokinins**

Mine Site Restoration = Facilitated (Natural) Succession?

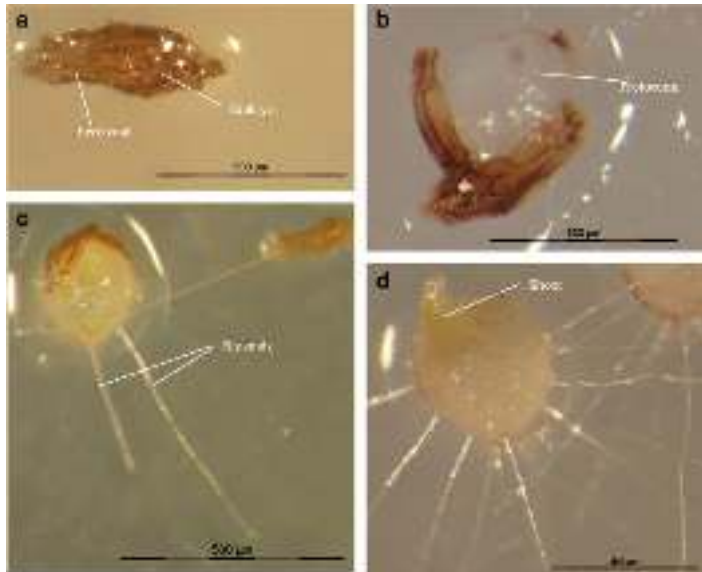
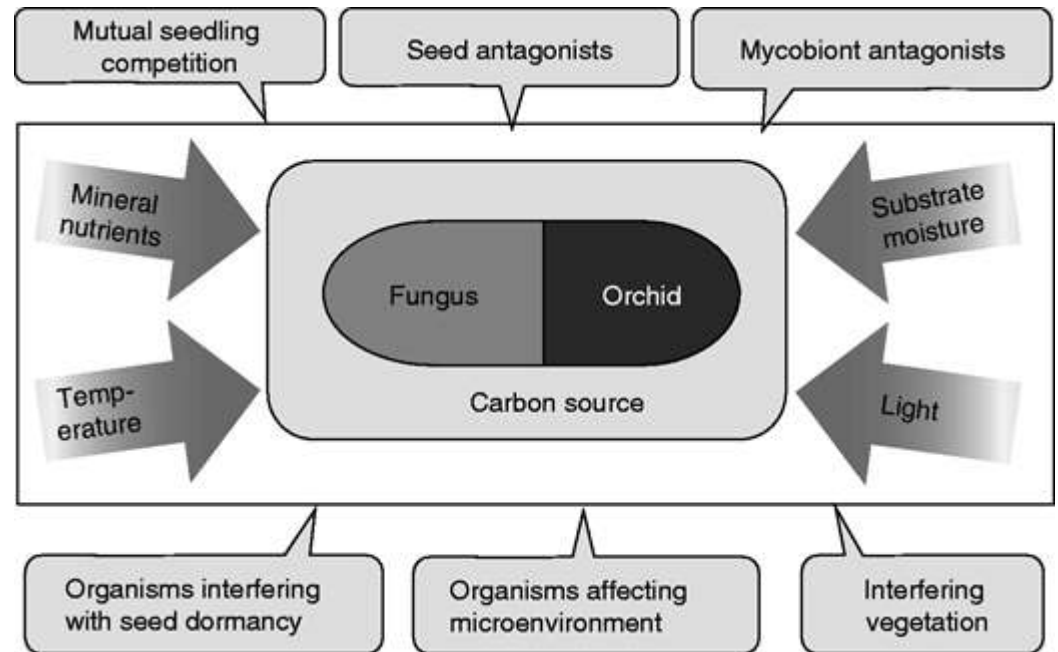


Ecological Restoration



**Goldfields,
WA, Australia**

Importance of Microbe-Plant interactions



Rasmussen, Dixon, et al (2015)

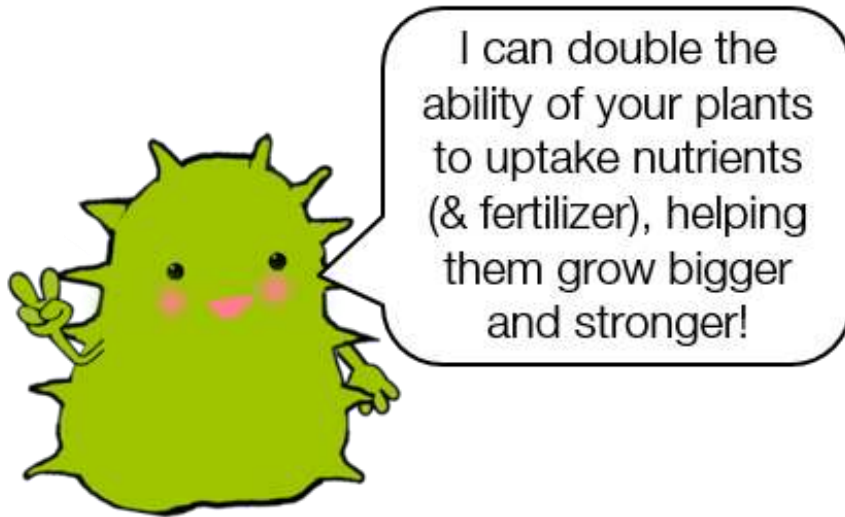


Cynorkis purpurea, a terrestrial
orchid

Rafter et al (2016)



Importance of Microbe-Plant interactions



What do the
Microbes “give” to the **Plants**?

Phytohormones help coordinate growth, development, & responses to stimuli

- **Phytohormones** are chemical signals that modify or control one or more specific physiological processes within a plant
- **Phytohormones** are produced in **very low concentration**, but a minute amount can greatly affect growth and development of a plant organ
- In general, **Phytohormones** control plant growth and development by affecting the division, elongation, and differentiation of cells
- They have **multiple, overlapping, & interacting functions**

Table 39.1

Table 39.1 Overview of Plant Hormones

Hormone	Where Produced or Found in Plant	Major Functions
AUXINS	Shoot apical meristems and young leaves are the primary sites of auxin synthesis. Root apical meristems also produce auxin, although the root depends on the shoot for much of its auxin. Developing seeds and fruits contain high levels of auxin, but it is unclear whether it is newly synthesized or transported from maternal tissues.	Stimulates stem elongation (low concentration only); promotes the formation of lateral and adventitious roots; regulates development of fruit; enhances apical dominance; functions in phototropism and gravitropism; promotes vascular differentiation; retards leaf abscission.
CYTOKININS	These are synthesized primarily in roots and transported to other organs, although there are many minor sites of production as well.	Regulate cell division in shoots and roots; modify apical dominance and promote lateral bud growth; promote movement of nutrients into sink tissues; stimulate seed germination; delay leaf senescence.
GIBBERELLINS	Meristems of apical buds and roots, young leaves, and developing seeds are the primary sites of production.	Stimulate stem elongation, pollen development, pollen tube growth, fruit growth, and seed development and germination; regulate sex determination and the transition from juvenile to adult phases.
BRASSINOSTEROIDS	These compounds are present in all plant tissues, although different intermediates predominate in different organs. Internally produced brassinosteroids act near the site of synthesis.	Promote cell expansion and cell division in shoots; promote root growth at low concentrations; inhibit root growth at high concentrations; promote xylem differentiation and inhibit phloem differentiation; promote seed germination and pollen tube elongation.
ABSCISIC ACID (ABA)	Almost all plant cells have the ability to synthesize abscisic acid, and its presence has been detected in every major organ and living tissue; may be transported in the phloem or xylem.	Inhibits growth; promotes stomatal closure during drought stress; promotes seed dormancy and inhibits early germination; promotes leaf senescence; promotes desiccation tolerance.
STRIGOLACTONES	These carotenoid-derived hormones and extracellular signals are produced in roots in response to low phosphate conditions or high auxin flow from the shoot.	Promote seed germination, control of apical dominance, and the attraction of mycorrhizal fungi to the root.
ETHYLENE	This gaseous hormone can be produced by most parts of the plant. It is produced in high concentrations during senescence, leaf abscission, and the ripening of some types of fruits. Synthesis is also stimulated by wounding and stress.	Promotes ripening of many types of fruit, leaf abscission, and the triple response in seedlings (inhibition of stem elongation, promotion of lateral expansion, and horizontal growth); enhances the rate of senescence; promotes root and root hair formation; promotes flowering in the pineapple family.
Karrikins		



Plant tissue cultures and Phytohormones

– **cytokinins** in coconut water



Dendrobium orchids



Liya Ge¹
Jean Wan Hong Yong¹
Swee Ngin Tan¹
Eng Shi Ong²

¹Natural Sciences and Science Education Academic Group, Nanyang Technological University, Singapore, Singapore
²Applied Science School, Temasek Polytechnic, Singapore, Singapore

Research Article

Determination of cytokinins in coconut (*Cocos nucifera* L.) water using capillary zone electrophoresis-tandem mass spectrometry

The applicability of CZE in combination with MS and MS/MS methods for the simultaneous separation and determination of 12 cytokinins was investigated for the first time. Cytokinins were first completely separated by CZE within less than 20 min using a

Liya Ge¹
Jean Wan Hong Yong¹
Swee Ngin Tan¹
Lin Hui²
Eng Shi Ong²

¹Natural Sciences and Science Education Academic Group, Nanyang Technological University, Singapore, Republic of Singapore
²Applied Science School, Temasek Polytechnic, Singapore, Republic of Singapore
³Department of Community, Occupational, and Family Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Republic of Singapore

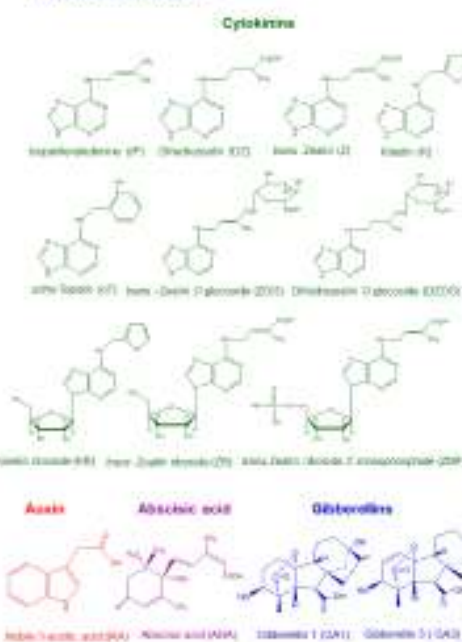
Research Article

Analyses of gibberellins in coconut (*Cocos nucifera* L.) water by partial filling-micellar electrokinetic chromatography-mass spectrometry with reversal of electroosmotic flow

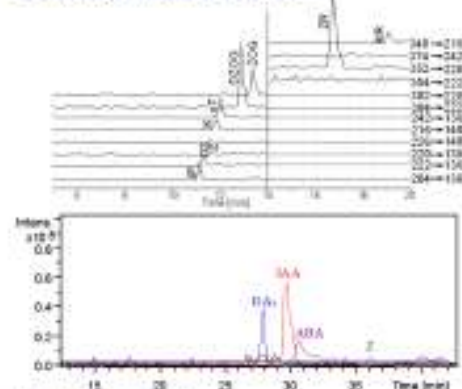
In this paper, we present the results of simultaneous estimating of eight gibberellins (GA) in coconut (*Cocos nucifera* L.) water by MEKC directly coupled to ESI-MS detection. During the development of MEKC-MS, partial filling (PF) was used to prevent the mistakes from meeting the mass spectrometer as this is detrimental to the MS signal, and a cathodic surfactant, octadecyltrimethylammonium hydroxide, was added to the electrolyte to reverse the EOF. On the basis of the resolution of the neighboring peaks, different po-

Several **Phytohormones** are found in **young coconut water** especially **cytokinins**, **auxins** and **gibberellins**.

Structures of endogenous phytohormones in coconut water^{1,2}



CE-MS/MS analytical results^{2,3}





**Mass
Micropropagation**





Bacterial cultures' cytokinin levels

Table IV. Concentration of Cytokinins in the Culture Supernatants of the Strains IC3342 and ANU240

The values in parentheses were determined by GC-MS; the other values were obtained by RIA.

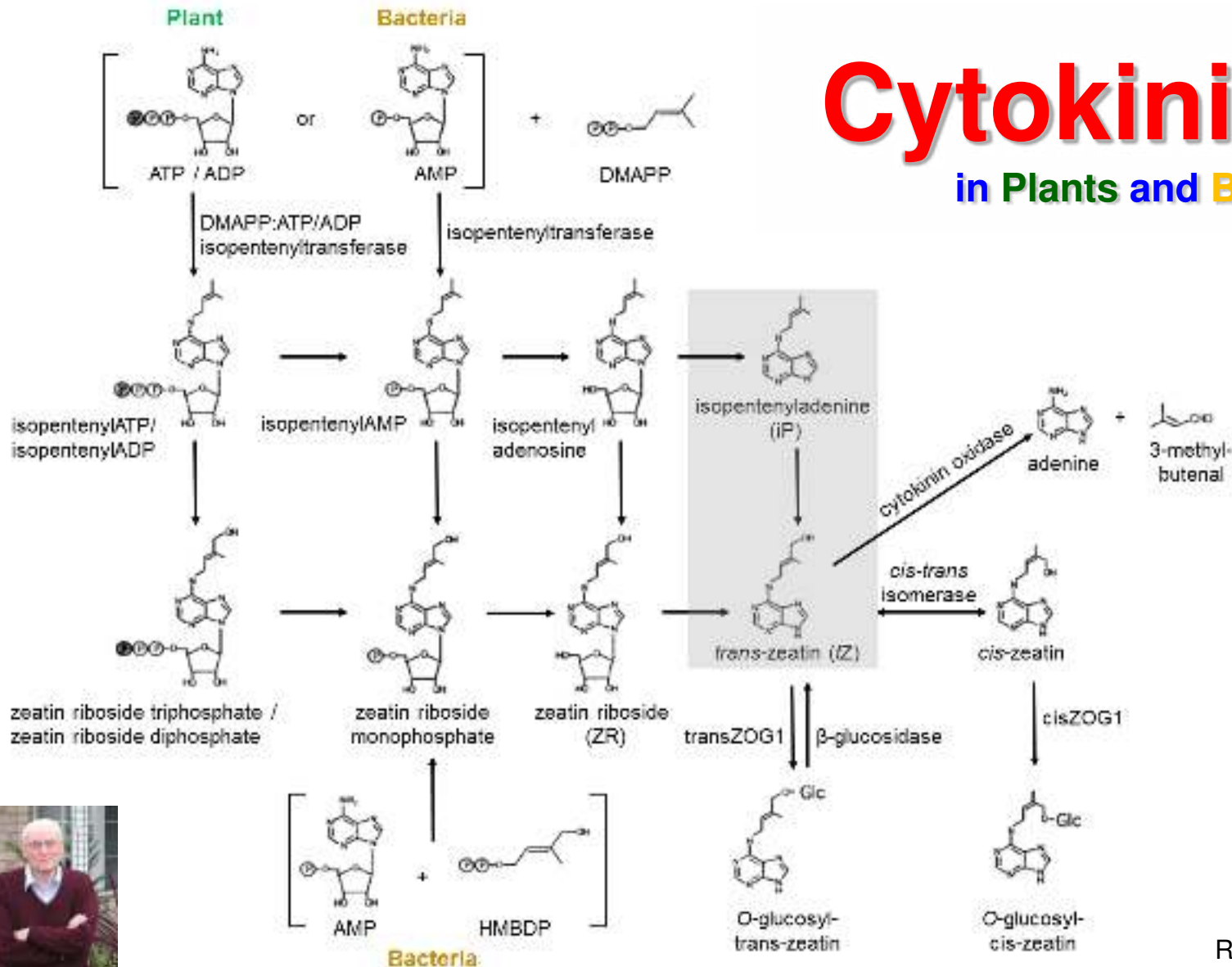
Cytokinins	Concentration in the Culture Supernatants of	
	ANU240	IC3342
	ng/L	
Z	83 (105)	2,188 (2,410)
DZ	54	133
ZR	9	7
DZR	8	9
cis-Z	166	176
cis-ZR	37	2
iP	5,191 (7,128)	41,420 (44,356)
iPA	123	329

Upadhyaya et al (1991)
Plant Physiology



Cytokinin types

in Plants and Bacteria



RIKEN Prof. H. Sakakibara



ANU Prof. Stuart Letham



Wong et al. (2015)
Springer

Figure 5. A model for cytokinins biosynthesis and metabolic pathway in plants and bacteria. In plants, the isopentenyl moiety from dimethylallyl diphosphate (DMAPP) is transferred to ATP/ ADP while the bacterial pathways starts off with AMP. Bacteria cytokinin biosynthesis may also start by transferring isopentenyl moiety from 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate (HMBDP) to AMP. Active cytokinins are shaded.

Adapted from: Frébart et al. (2011); Haberer and Kleber (2002); Kakimoto (2003); Sakakibara (2006) and Tarkowski et al. (2009).

Current Trends



Liquid biofertilizers or “Biostimulants”

Steady-state

Past Successful Track Record



Ongoing

Fundamental Question:

Why add **“HORMONE”** to a standard NPK and TE (trace elements) chemical fertilizer mixture?



Is this becoming more common in the Agri-Horti industries?

Is Fish Waste a **GOOD** Fertilizer?

- Measure the essential nutrients (Macro & micro nutrients, trace elements)
- Determine the **natural biostimulants** in the **fish waste sludge** and **aquaponics**.





Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Aquaculture 230 (2004) 125–135

Aquaculture

www.elsevier.com/locate/aqua-online

Performance evaluation of rice–fish integration system in rainfed medium land ecosystem

Rajeeb K. Mohanty^a, H.N. Verma, P.S. Brahmanand

*Water Technology Centre for Eastern Region, Indian Council of Agricultural Research (ICAR),
APPO-Chandrasekharpur, Bhubaneswar 751023, India*

Received 22 October 2002; received in revised form 13 May 2003; accepted 15 May 2003

An experiment in India indicated a 4.9–8.6% increase in rice yield under rice–fish culture, and the increase was associated with an increased number of panicles and an increased number of filled grains per panicle (Mohanty et al., 2004).



Rice yield
increased
4.9 to 8.6 %
with **fishes**

Ashikari et al (2005) Science

Connections to a typical **Cytokinin** effect!



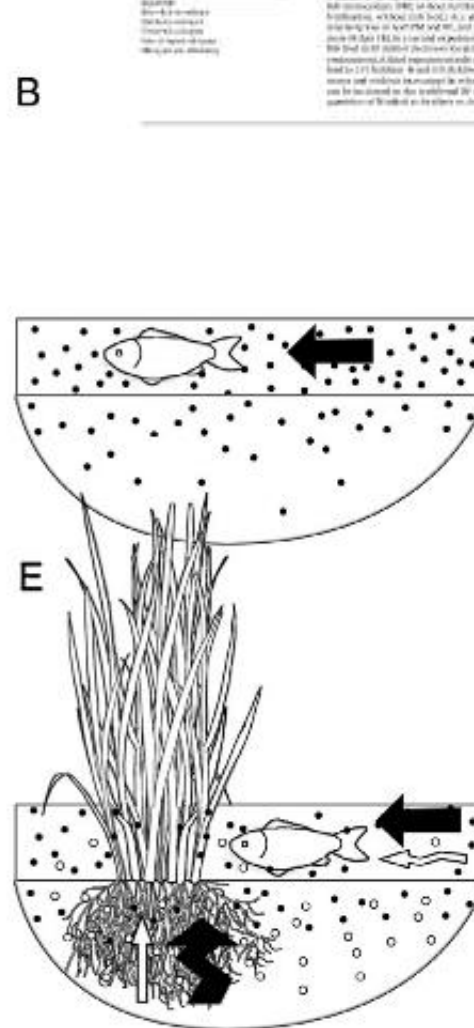
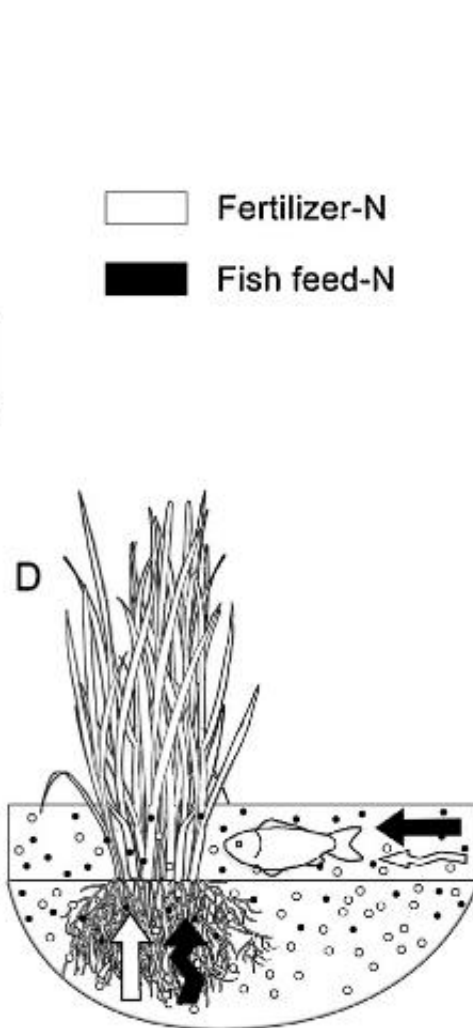
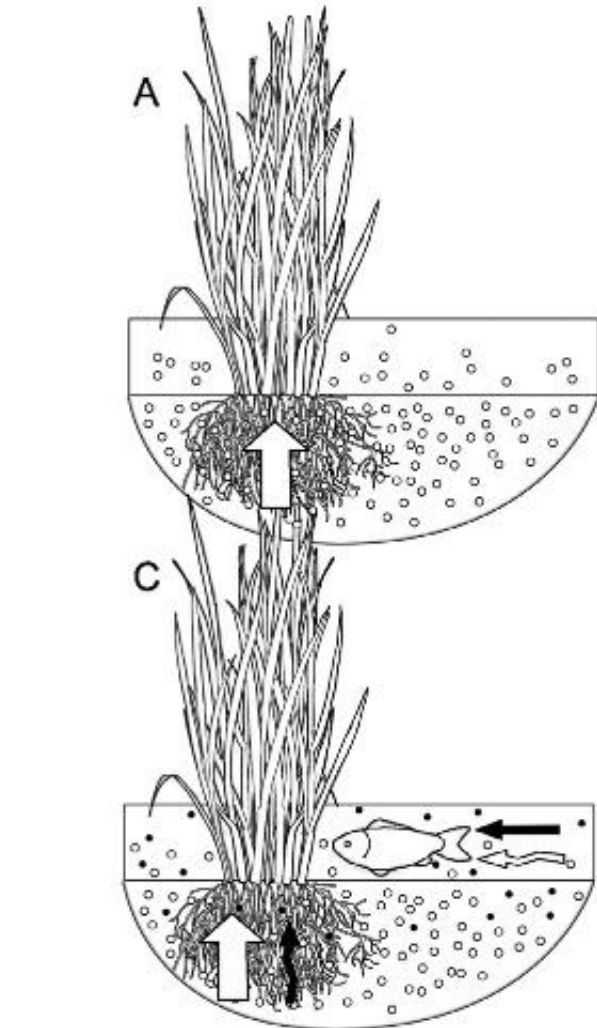




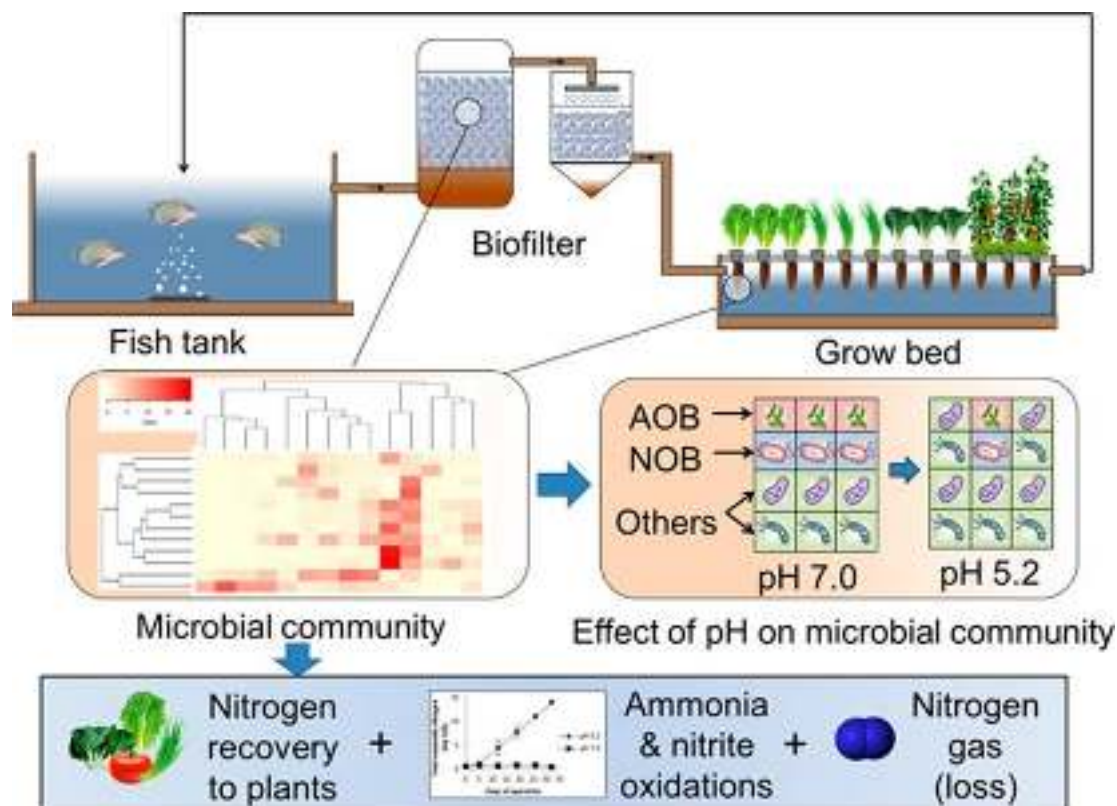
Table 1
Yields of rice and fish in the three experiments.

Experiments and treatments	Rice yield (tha ⁻¹)		Fish yield (kg ha ⁻¹)	
	Grain	Straw	Gross	Net
Experiment 1				
RM	6.19 ± 0.17a	4.42 ± 0.12a		
FM			414.49 ± 37.53a	302.49 ± 37.53a
RF	6.29 ± 0.26a	4.43 ± 0.18a	484.45 ± 70.90a	372.45 ± 70.90a
Experiment 2				
RM	4.87 ± 0.38a	3.50 ± 0.24a		
FM60			1274.75 ± 90.65b	1163.06 ± 90.65b
RF30	4.97 ± 0.38a	3.58 ± 0.32a	685.75 ± 54.38c	629.91 ± 54.38c
RF60	5.20 ± 0.43a	3.79 ± 0.52a	1190.75 ± 104.43b	1079.06 ± 104.43b
RF90	5.22 ± 0.40a	3.75 ± 0.25a	1578.25 ± 243.85b	1410.72 ± 243.85b
RF120	4.98 ± 0.23a	3.59 ± 0.23a	2159.00 ± 199.54a	1935.62 ± 199.54a
Experiment 3				
RF25%	5.67 ± 0.09a	3.84 ± 0.10a	655.62 ± 75.08b	338.37 ± 70.71b
RF44%	5.36 ± 0.12a	3.49 ± 0.24a	829.01 ± 34.84ab	516.26 ± 37.22ab
RF63%	5.37 ± 0.29a	3.21 ± 0.07a	1024.20 ± 73.82a	689.65 ± 79.07a

Values are means ± S.E. Numbers with different letters are statistically different based on LSD ($P \leq 0.05$). In experiment 1: RM (rice monoculture), RF (rice–fish co-culture), and FM (fish monoculture). In experiment 2: RM (rice monoculture), FM60 (fish monoculture, 60 fish plot⁻¹); RF30, RF60, RF90, and RF120 (rice–fish co-culture with 30, 60, 90, and 120 fish plot⁻¹, respectively). In experiment 3: RF25% (RF with 75% fertilizer-N and 25% feed-N); RF44% (RF with 56% fertilizer-N and 44% feed-N); RF63% (RF with 37% fertilizer-N and 63% feed-N).

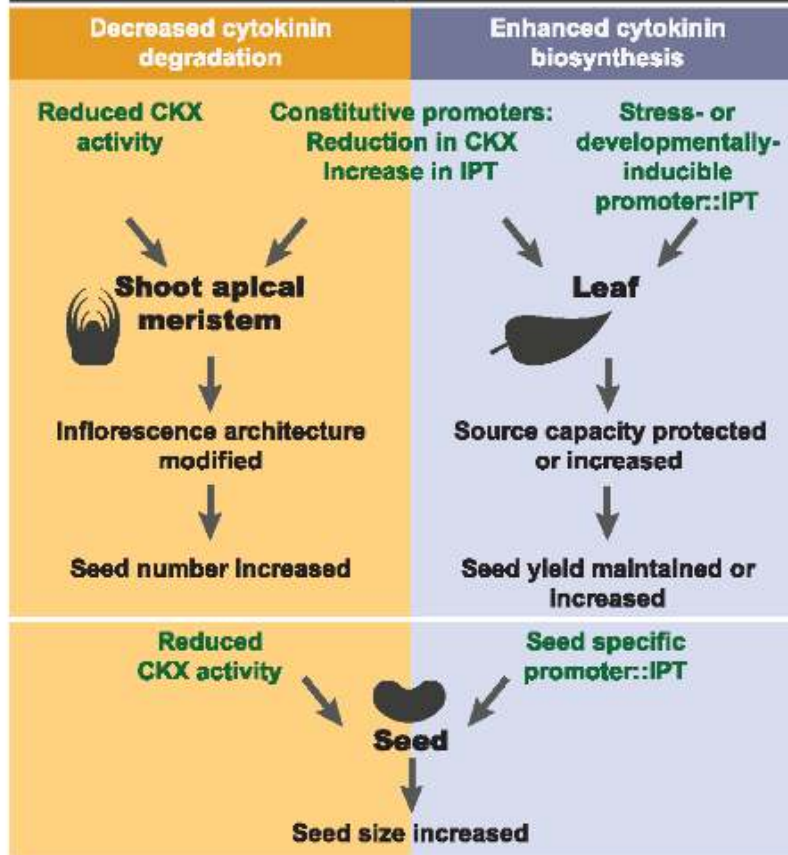


Aquaponics



A better experimental system to measure natural **Biostimulants** in animal waste?

Effect on seed yield of modifying cytokinin content in leaf, shoot apical meristem and seed



Cytokinin Oxidase Regulates Rice Grain Production

Motoyuki Ashikari,^{1*} Hitoshi Sakakibara,^{2*} Shaoyang Lin,³
Toshio Yamamoto,³ Tomonori Takashi,³ Asuka Nishimura,³
Enrique R. Angeles,³ Qian Qian,⁴ Hidemi Kitano,¹
Makoto Matsuoka^{1†}

Most agriculturally important traits are regulated by genes known as quantitative trait loci (QTLs) derived from natural allelic variations. We here show that a QTL that increases grain productivity in rice, *Gn1a*, is a gene for cytokinin oxidase/dehydrogenase (*OsCKX2*), an enzyme that degrades the phytohormone cytokinin. Reduced expression of *OsCKX2* causes cytokinin accumulation in inflorescence meristems and increases the number of reproductive organs, resulting in enhanced grain yield. QTL pyramiding to combine loci for grain number and plant height in the same genetic background generated lines exhibiting both beneficial traits. These results provide a strategy for tailor-made crop improvement.

Journal of Experimental Botany, Vol. 87, No. 3 pp. 593–606, 2016
doi:10.1093/jxb/erv461 Advance Access publication 1 November 2015

REVIEW PAPER

Cytokinin: a key driver of seed yield

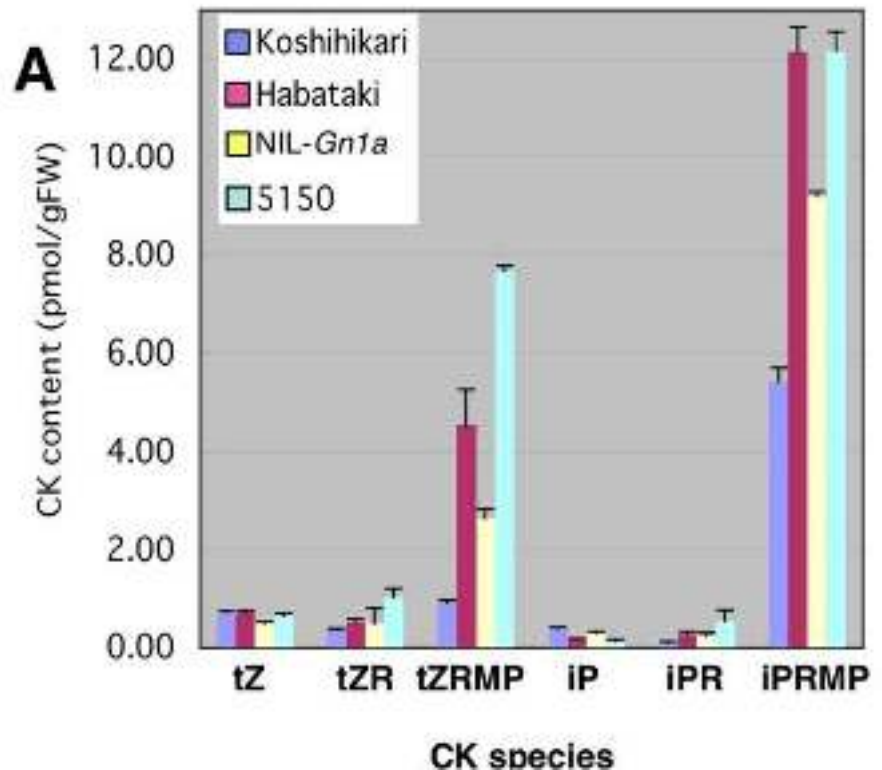
Paula Elizabeth Jameson^{1,*} and Jiancheng Song^{1,2}

¹ School of Biological Sciences, University of Canterbury, Christchurch 8140, New Zealand

² School of Life Sciences, Yantai University, Yantai 264005, China

* To whom correspondence should be addressed. E-mail: Paula.Jameson@canterbury.ac.nz

Higher cytokinin levels (within limits),
more grains



Ashikari et al (2005) Science

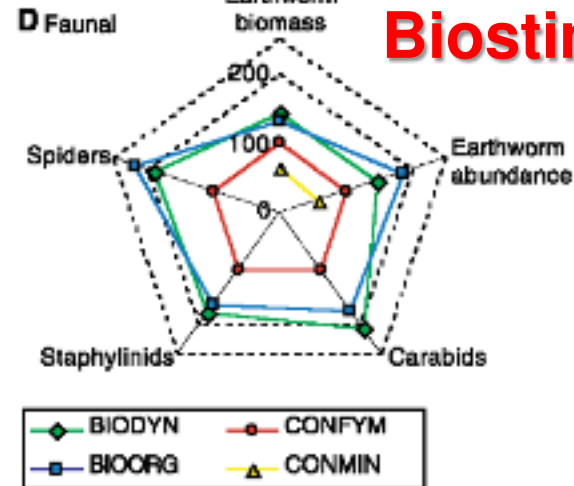
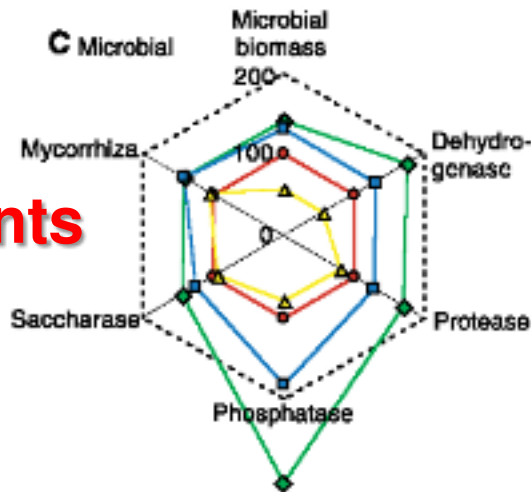
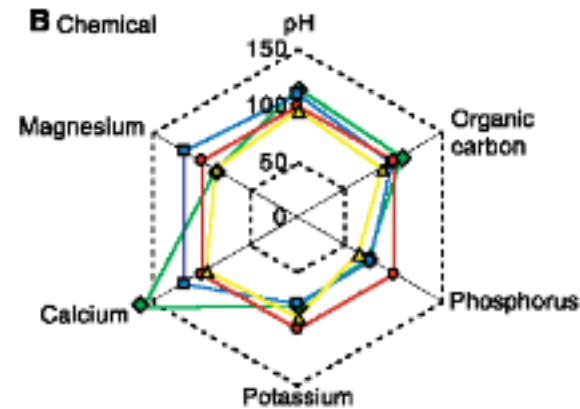
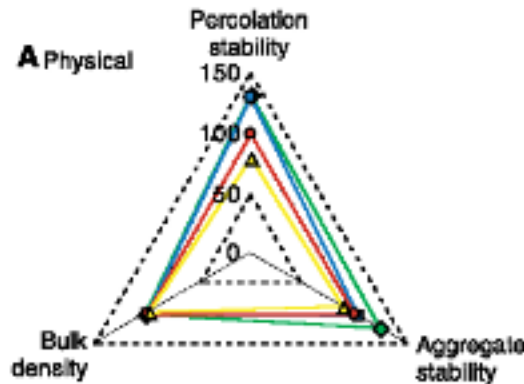
Bridging the divide between **Conventional** & **Organic** mode of cultivation

A-NPK

A refers to the **A**ctive ingredients or **A**ctive compounds present in any given fertilizers/soils.

A is not just any phytohormones like cytokinins, but including the other phytohormones like auxins, gibberellins, karrikins, etc., MicroProteins, protein hydrolysates, and their stabilizing (“chelating”) associates like humic and fulvic acids, etc.

Organic plant production



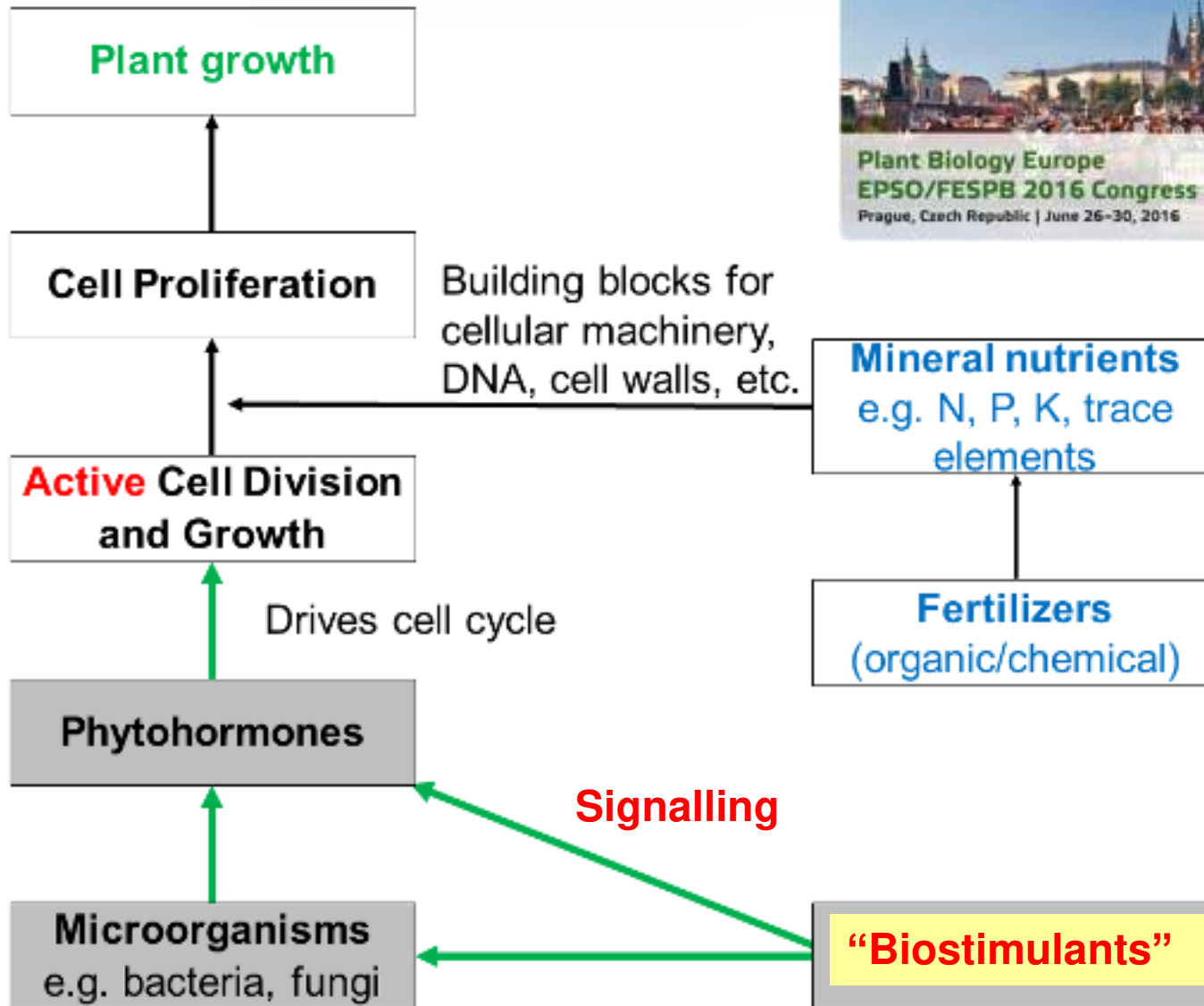
Biostimulants

Biostimulants

Mäder et al (2002) Science

Figure 2 Physical, chemical, and biological soil properties in soils of the DOK farming systems. Analyses were done within the plough horizon (0 to 20 cm) except for soil fauna. Results are presented relative to CONFYM (= 100%) in four radial graphs. Absolute values for 100% are as follows. (A) Percolation stability, 43.3 ml min⁻¹; aggregate stability, 55% stable aggregates > 250 µm; bulk density, 1.23 g cm⁻³. (B) pH(H₂O), 6.0; organic carbon, 15.8 g C_{org} kg⁻¹; phosphorus, 21.4 mg P kg⁻¹; potassium, 97.5 mg K kg⁻¹; calcium, 1.7 g Ca kg⁻¹; magnesium, 125 mg Mg kg⁻¹. (C) Microbial biomass, 285 mg C_{mic} kg⁻¹; dehydrogenase activity, 133 mg TPF kg⁻¹ h⁻¹; protease activity, 238 mg tyrosine kg⁻¹ h⁻¹; alkaline phosphatase, 33 mg phenol kg⁻¹ h⁻¹; saccharase, 526 mg reduced sugar kg⁻¹ h⁻¹; mycorrhiza, 13.4% root length colonized by mycorrhizal fungi. (D) Earthworm biomass, 183 g m⁻²; earthworm abundance, 247 individuals m⁻²; carabids, 55 individuals; staphylinids, 23 individuals; spiders, 33 individuals. Arthropods have not been determined in the CONMIN system because of the field trial design. Significant effects were found for all parameters except for bulk density, C_{org}, and potassium (analysis of variance; *P* < 0.05). For methods, see table S3.

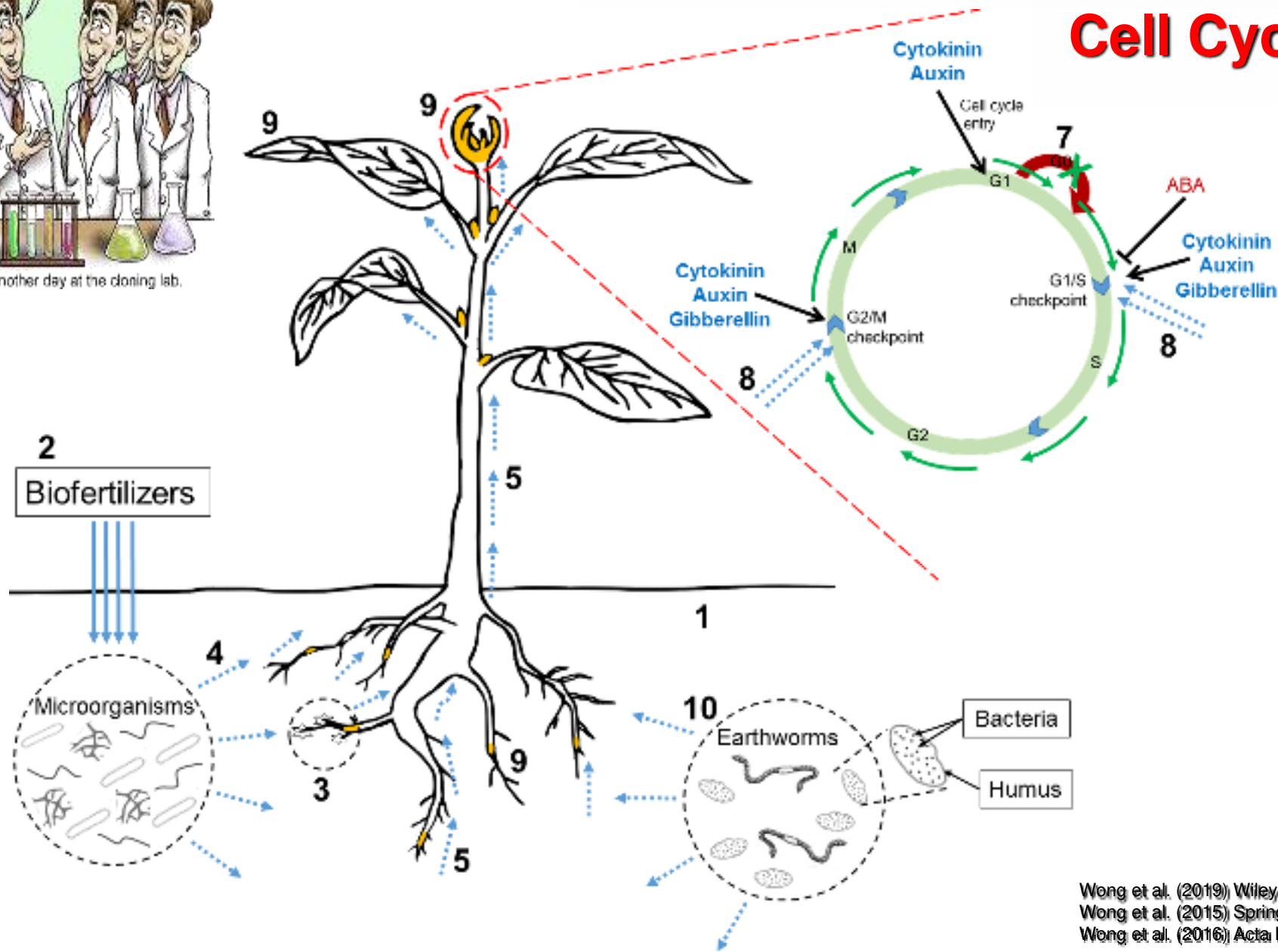
New paradigm for Plant Nutrition





Conceptual Model

Cell Cycle

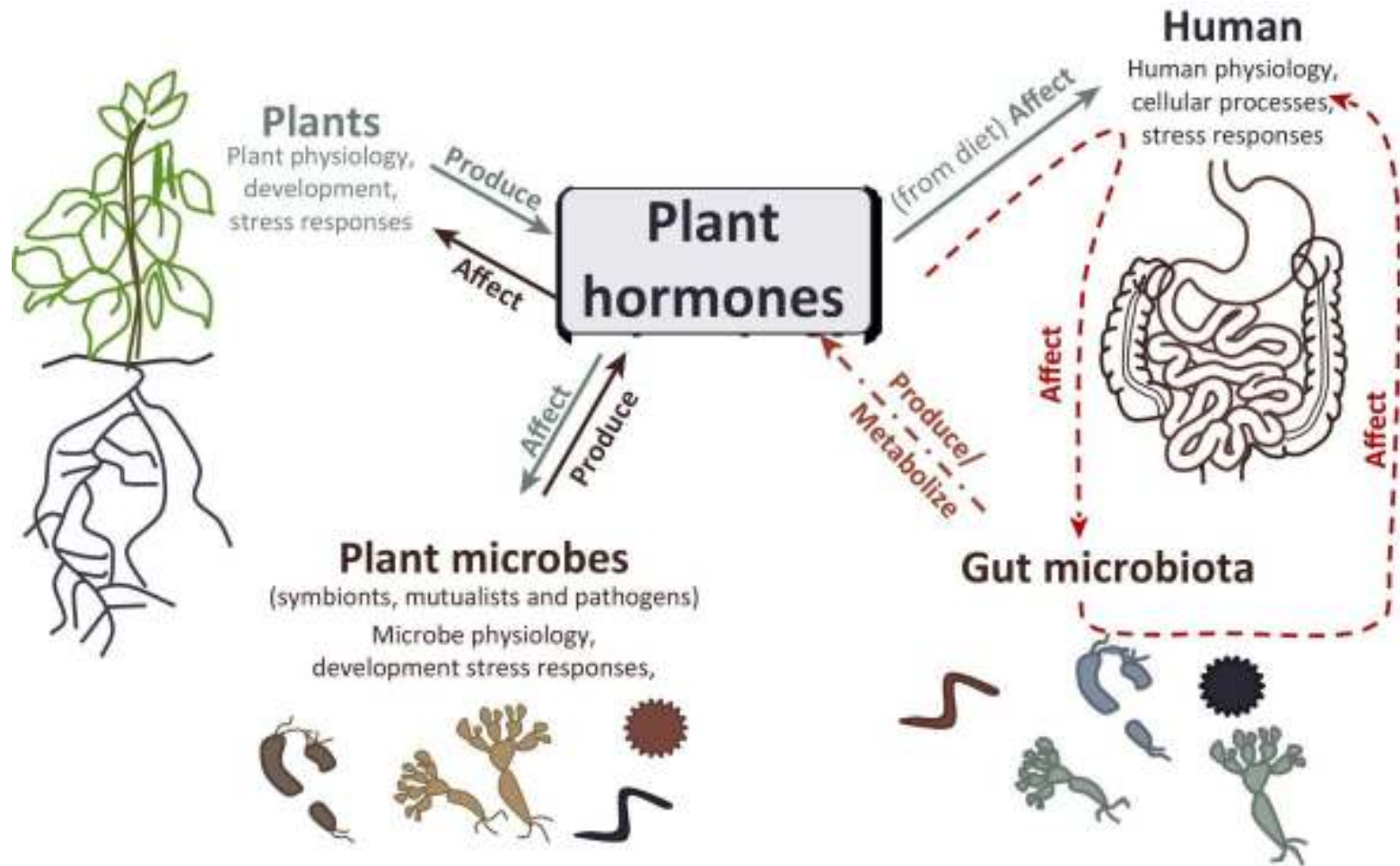


What is a **GOOD** Fertilizer?

- Essential nutrients (Macro & micro nutrients, trace elements)
- Improves soil structure & holds water
- **Promote plant cell division leading to cell proliferation** (hormones, etc - biostimulants)

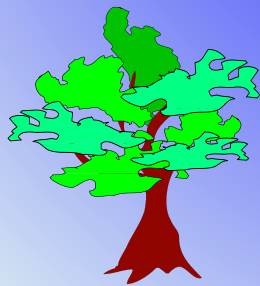


Increasing body of evidence to show that **phytohormones** affect **human health** through the **diet** and **microbes**



Trends in Plant Science

“The true voyage of discovery lies not in finding new landscapes, but in having new eyes.”



- Marcel Proust

Thank You!

Hortikulturell produktionsfysiologi (HPF),
Institutionen för biosystem och teknologi

Horticultural Production Physiology,
Department of Biosystems and Technology



Swedish University of
Agricultural Sciences



Acknowledgments



**ARC Centre for
Mine Site Restoration**

An Industrial Transformation Training Centre



THE UNIVERSITY OF
**WESTERN
AUSTRALIA**

Curtin 
University of Technology

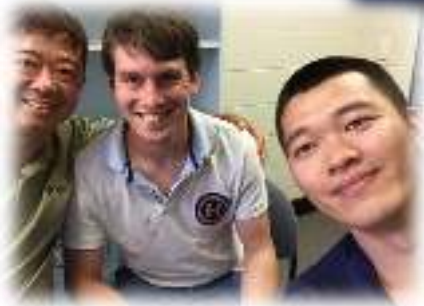
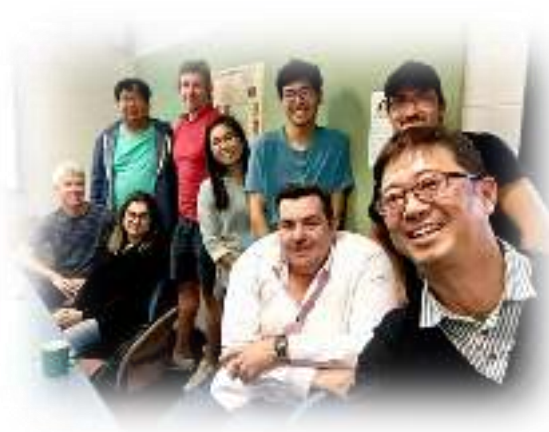


Australian Government

Australian Research Council



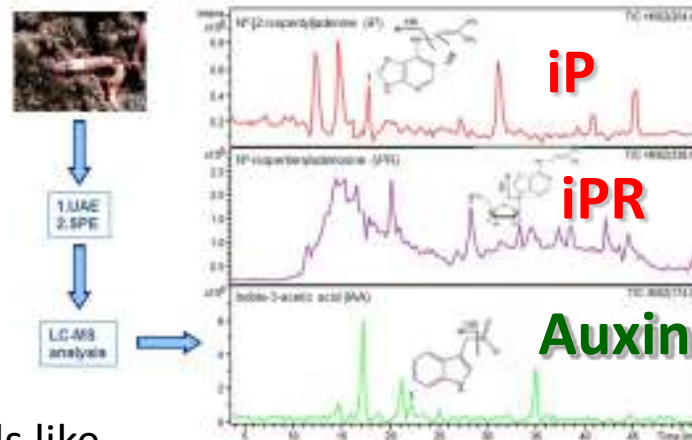
Thank You!



Multi-disciplinary & collaborative research
give significant cost-savings!!!
Insightful research outcomes – better papers too



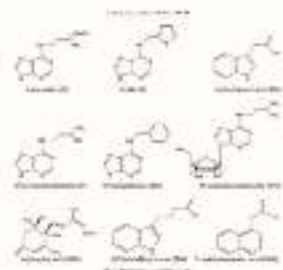
Advanced “analytical tools”



New tools like
**Soil Biological
Functionality
Assessment &
Rhizosphere
Competency**



GCMS



UPLC

GCMS

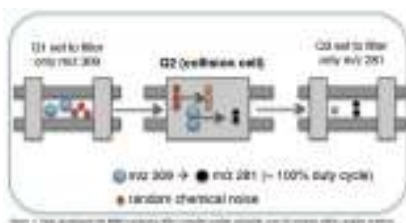
ELISA & SPA



Note SPA: Scintillation Proximity Assay



LCMS/MS



**Triple-
Quad
LCMS**



Effects of chemical fertilizers or/and organic fertilizers on **Tomato** photosynthesis



Swedish University of
Agricultural Sciences

Cell Cycle Control & Cytokinins

Planta (1996) 200: 2–12

Planta
© Springer-Verlag 1996

Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of p34^{cdc2}-like H1 histone kinase

Kerong Zhang¹, David S. Letham^{1,2}, Peter C.L. John^{1,2}

¹ Plant Cell Biology Group, Research School of Biological Sciences, The Australian National University, ACT 2601, Australia

² Cooperative Research Centre for Plant Science, GPO Box 475, Canberra, ACT 2601, Australia

Received: 27 October 1995/Accepted: 8 January 1996

Abstract. In excised pith parenchyma from *Nicotiana tabacum* L. cv. Wisconsin Havana 38, auxin (naphthalene-1-acetic acid) together with cytokinin (6-benzylamino-purine) induced a greater than 40-fold increase in a p34^{cdc2}-like protein, recoverable in the p13^{vas1}-binding fraction, that had high H1 histone kinase activity, but enzyme induced without cytokinin was inactive. In suspension-cultured *N. plumbaginifolia* Viv., cytokinin (kinetin) was stringently required only in late G2 phase of the cell division cycle (cdc) and cells lacking kinetin arrested in G2 phase with inactive p34^{cdc2}-like H1 histone kinase. Control of the Cdc2 kinase by inhibitory tyrosine phosphorylation was indicated by high phosphotyrosine

Introduction

Cell cycle control can be exercised by interaction of the key cell division cycle (cdc) catalyst p34^{cdc2} (the 34-kDa product of the *cdc2* gene) with different cyclin subunits that direct its protein kinase activity to specific substrates (Peeper et al. 1993), by cyclin dependent kinase inhibitor (CKI) proteins (Pines 1995) and by enzymes that control its enzyme activity through phosphorylation (Gould and Nurse 1987; Millar et al. 1991), so providing a likely universal mitotic control (Nurse 1990).

In plants we have noted changes in the level of p34^{cdc2}-like protein that are consistent with an hypothesis





ANU Graham Farquhar & Chin Wong

Importance of Roots-derived Phytohormones

Using a **Root-pressure chamber** to obtain “snap-shots” of xylem sap for various analyses.

Yong et al (2000, 2010)





Rice-soft shell turtle coculture effects on yield and its environment

Jian Zhang^a, Liangliang Hu^a, Weizheng Ren^a, Liang Guo^a, Jianjun Tang^a, Miaoan Shu^{b,*}, Xin Chen^{b,*}^a College of Life Sciences, Zhejiang University, Hangzhou 310058, China^b College of Animal Sciences, Zhejiang University, Hangzhou 310058, China**Table 1**

Turtle and rice yields in a 2-year field experiment with rice monoculture (RM), rice-turtle coculture (RT), and turtle monoculture (TM) in China. The turtle is *Pelodiscus sinensis*.

	Treatment		
	RM	RT	TM
Yields			
Rice (ton ha ⁻¹)			
Grain yield	7.67 ± 0.15 ^a	7.46 ± 0.26 ^a	
Straw yield	4.67 ± 0.04 ^b	4.98 ± 0.09 ^a	
Turtle (ton ha ⁻¹)			
Gross yield		1.56 ± 0.01 ^a	1.48 ± 0.03 ^a
Net yield		1.15 ± 0.01 ^a	1.09 ± 0.03 ^a

Values are means ± SE (n=4). Means in a row followed by different letters are significantly different (P < 0.05).

**TURTLE POO**

for

rice

cultivation!

Chinese soft-shelled turtle (*Pelodiscus sinensis*)