





Hortikulturell produktionsfysiologi (HPF), Institutionen för biosystem och teknologi



Horticultural Production Physiology, **Department of Biosystems and Technology**







SLU

















Understanding the Role of Biostimulants in Cultivation















Swedish University of Agricultural Sciences



Hortikulturell produktionsfysiologi (HPF), Institutionen för biosystem och teknologi





Learning Outcome



What is a GOOD "Fertilizer"?

- Essential nutrients (Macro - NPK & micro-nutrients as building blocks)
- Improves soil structure & holds water







Who am I?





T-Shaped Skills

I am a T-shaped Scientist



Phalaenopsis potted-flower production

Control of Flowering

Sympodial Orchids

Monopodial tropical orchids

Control of Flowering Agribusiness –

Orchid In-Vitro flowering





















Sim et al (2007)



(0)



ANU Graham Farquhar & Chin Wong



(a)

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, 2014)

Basic Research





How to connect the different disciplines?

How did I arrived at the understanding of Biostimulants?



Orchid Industry & Conservation



Healthy Soils

"Accidental" soil scientist!

Mid-West Western Australia, Australia

Applied Research WESTERN AUSTRALIA

64

2018







Iron Ore Mining

Tailings

Operations

Top soil storage area

Waste Rock trial planting

Mid-West Western Australia, Australia



Sand Mining

Elisabrook, near Perth, Australia

Australia, 2017-2018



Coldie

Ecological Restoration

Ricinocarpos brevis

Large-scale Biostimulants' usage & Soil Re-constitution



: Lampung, Indonesia : 33 000 ha

Location

Total Area



Lampung, Sumatra, Indonesia

Organic Transformation

Consultancy

PT Great Giant Pineapple

Case Study : Great Giant Pineapple





Conceptual Connections



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Eichmann et al. 2021



Complexity of Plants' responses

Abiotic vs Biotic factors



Pozo et al. 2015 (New Phyto)

Traditional, Organic/Green Fertilizers

- Composts, Vermi-compost (earthworms), Frass
- Humic substances
- Liquid/pellet microbial inoculants

Biostimulants

Plant biostimulants are <u>diverse substances</u> and <u>microbes</u> used to <u>enhance</u> growth, *independent* of the mineral nutritional effects.





Formal Definition Plant Biostimulants in FPR

DEFINITION

"A plant biostimulant shall be an EU fertilising product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere:

- (a) nutrient use efficiency,
- (b) tolerance to abiotic stress,
- (c) quality traits, or
- (d) availability of confined nutrients in the soil or rhizosphere."



Dr Theodora Nikolakopoulou, EU Commission

FPR: Fertilizer Product Registration



Biostimulants – where to find it?



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Huge Diversity of "Compounds"

Plant biostimulants New tools in the grower's toolbox

Jordbruk

1012000







Biostimulants

NILEY SERIES IN RENEWARI E RESOURCES

The Chemical

Plant Biostimulants

Biology of

Biostimulants – why use it?



Van Ittersum & Rabbinge (1997) Field Crops Research





Biostimulants function like "Catalyst"



Defining the Organics

Circular Economy



Biostimulants

Eight categories (Calvo et al. 2014)

- Microbial inoculants (bacteria, cyanobacteria, fungi)
- 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) Marschner Review



Plant Soil (2014) 383:3-41 DOT 10 1007/s11104_014.2131.8 MARSCHNER REVIEW Agricultural uses of plant biostimulants Pamela Calvo - Louise Nelson - Joseph W. Kloepper Received: 20 December 2013 / Accepted: 25 April 2014 / Published online: 8 May 2014 (0) The Author(s) 2014. This article is published with open access at Springerlink.com Abstract Keywords Microbial moculants -Humic acid -Fulyac Background Plant biostimulants are diverse substances acid · Protein hydrolysates · Amino acids · Seaweed and microorganisms used to enhance plant growth. The extracts · Biostimulants global market for biostimulants is projected to increase 12 % per year and reach over \$2,200 million by 2018. Despite the growing use of biostimulants in agriculture, Introduction many in the scientific community consider biostimulants to be lacking peer-reviewed scientific Plant biostimulants, or agricultural biostimulants, inevaluation

Scope This article describes the emerging definitions of biostimulants and reviews the literature on five catego-

Plant biostimulants, or agricultural biostimulants, include diverse substances and microorganisms that enhance plant growth. The global market for biostimulants has been projected to reach \$2,241million by 2018 and

The Chemical Biology of Plant Biostimulants



Phytohormones – old timers and newcomers



AN INNOVATION FROM THE PLANT CELL

ideas to grow on

© 2011 American Society of Plant Biologists

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*¹

*School of Integrative Biology, [†]ARC 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

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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 matter.

PNAS

soil p

(13), (ii) most other heathland plants have mycorrhizal symbioses and/or form symbioses with N_2 -fixing microbes (13), and



roots than plants grown without nitrogen (Fig. 1A and B). Shoot

bic bic wit SLU Plant Protein Factory

ogen

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was observed with morganic nitrogen (Fig. 1 A and B). Arabidopsis grown with protein (1.5 or 6 mg BSA per ml of growth modium) as the sele pitrogen source had significantly areator dry

nitrogen untake Lorganic nitrogen Lolant nutrition Lolant roots L

New Ideas, Original Thinking?

microbes and animals (1, 2). Despite the importance of protein in soils, little research has been carried out to elucidate the role **DWINGO** en as an itrogen source for plants. The where the rate of microbial mineralization is slowed by a sin boreal forests and heathlands, woody plants rely on ecto- or ericoid mycorrhizal fungal symplants grown with 6 mg BSA per ml growth medium. Arabidopsis supplied with resixture of protein and a low count of inorganis nitrogen (5, m) is the character of the only of the progrowth medium) growth mean ly before the only of growthin either nitrogen source individually and produced the same dry weight as plants grown with a high amount of inorganic nitrogen

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



Phytohormones is in µg to ng



Movement of Plants' Signalling Substances









Complexity of Plants' responses

Gibberellins



Pozo et al. 2015 (New Phyto)

Gibberellins (GA)

 Gibberellins have a variety of effects, such as stem elongation, fruit growth, and seed germination.







Nitrogen-responsive chromatin modulation enhances rice tillering.



Kun Wu et al. Science 2020;367:eaaz2046



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Cell Cycle Control & Cytokinins

Planta (1996) 200: 2-12

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

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Received: 27 October 1995/Accepted: 8 January 1996

Abstract. In excised pith parenchyma from Nicotiana tabacum L. ev. Wisconsin Havana 38, auxin (naphthalenel-acetic acid) together with cytokinin (6-benzylaminopurine) induced a greater than' 40-fold increase in a $p34^{adc2}$ -like protein, recoverable in the $p13^{uucl}$ -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 phosnhorylation was indicated by high phosphotyrosine

Introduction

Cell cycle control can be exercised by interaction of the key cell division cycle (cdc) catalyst p34^{odc2} (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





D. Stuart Letham



(A)

Kinetin







(C)

N⁶- Benzyladenine

Cytokinins 细胞分裂素



Cytokinins form a major group of **Biostimulants**



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¹Natural Sciences and Science Education Academic Group. Nanyang Technological University, Singapore, Singapore ²Applied Science School, Temasek Polvtechnic. 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 Electrophores is 2008, 29, 0000-0000

Liya Ge¹ Jean Wan Hong Yong¹ Swee Ngin Tan¹ Lin Hua² Eng Shi Ong³

¹Natural Sciences and Science Education Academic Group, Nanyang Technological University, Singapore, Republic of Singapore ²Genome Institute of Singapore, Biopolis, Singapore, Republic of Singapore ³Department of Community, Occupational, and Family Medicine, Yong Loo Ling 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 screening of eight gibberellins (GAs) 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 micelles from reaching the mass spectrometer as this is detrimental to the MS signal, and a cationic surfactant, cetyltrimethylammonium hydroxide, was added to the electrolyte to reverse the EOF. On the basis of the resolution of the neighboring peaks, different pa-

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

2171





Naturally-occurring phytohormones identified in coconut water1-6

Phytohormones		Estimated original concentration (×10 ⁻³ µM)	
Cytokinins	isopentenyladenine	0.26	
	dihydrozeatin	0.14	
	trans-zeatin	0.09	
	kinetin	0.31	
	ortho-topolin	3.29	
	dihydrozeatin O-glucoside	46.6	
	trans-zeatin O-glucoside	48.7	
	trans- zeatin riboside	76.2	
	kinetin riboside	0.33	
	trans-zeatin riboside-5'- monophosphate	10.2	
	14-0- (3-0- [β-D- galactopyranosyl - (12) - α-D- galactopyranosyl - (13) - (13) -α-L- arabinofuranosyl] -β-D- galactopyranosyl) -β-D- galactopyranosyl) - rans- zeatin riboside	Present	
Gibberellins	gibberellin 1	16.7	
	gibberellin 3	37.8	
Auxin	indole-3-acetic acid	150.6	
Abscisic	Abscisic acid	65.5	







Natural Soil Fertility

Earthworms (vermi-compost)

AND BURGLUP

Perionyx excavatus and Eisenia foetida at 65:35 ratio

Earthworms' "effects"

A meta-analysis on the effects of earthworm presence on plant biomass



Vermicomposts



Zhang et al. 2015, Talanta

UAE - Ultra-Sound Assisted Extraction

Vermicompost tea – Other Research Groups

Plant Growth Regul (2015) 75:483-492 DOI 10.1007/s10725-014-0011-0

ORIGINAL PAPER

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

Adeyemi O. Aremu · Wendy A. Stirk · Manoj G. Kulkarni · Danuše Tarkowská · Veronika Turečková · Jiří Gruz · Michaela Šubrtová · Aleš Pěnčík · Ondřej Novák · Karel Doležal · 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 Cytokinins, auxins, abscisic acid, gibberellins (GAs) 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. N⁶-isopentenyladenine formed the major (60 %) proportion of the CK content while dihydrozeatin had the lowest (<0.02 %) concentration. Indole-3-acetic acid ranged from approximately 0.55-0.77 pmol/mL. A total of 18 GAs including bioactive forms and metabolic end products were observed in the VCL samples. Cathasterone had the highest (2,500-3,200 fg/mL) concentration while brassinolide was the lowest (1-5 fg/mL) abundant BRs found. Phenolic acids quantified were protocatechnic acid (3-3.6 µg/mL). p-hydroxybenzoic acid (2.5-2.8 µg/mL), p-coumaric acid (1-1.7 µg/mL) and ferulic acid (0-4 µg/mL). These results provide an indication of the rich diversity in natural PGRs and phytochemicals in VCL which may inevitably contribute to the numerous favorable physiological responses elicited by VCL application to plants.

Keywords Abscisic acid - Biostimulant Brassinosteroids · Cytokinins · Gibberellins · Phenolics

Abbreviations

Abscisic acid ABA BRs Brassinosteroids CKs Cytokinins cZ cis-Zeatin cZ9G cis-Zeatin-9-glucoside cZOG. cis-Zeatin-O-glucoside cZR cis-Zeatin riboside **cZRMP** cis-Zeatin riboside-5'-monophosphate cZROG cis-Zeatin-O-glucoside riboside DHZ Dihydrozeatin DHZ9G Dihydrozeatin-9-glucoside DHZOG Dihydrozeatin-O-glucoside DHZR Dihydrozeatin riboside DHZRMP Dihydrozeatin riboside-5'-monophosphate DHZROG Dihydrozeatin-O-glucoside riboside GAs Gibberellins IAA Indole-3-acetic acid LAC Immunoaffinity chromatography

In addition to Auxins and **Cytokinins**

+ Gibberellins + Brassinosteroids





Is Fish Waste a GOOD Fertilizer?



The levels of Biostimulants in Fish Sludge as determined by LC-MS/MS

	Biostimulant	Concentration (pmol gDW)	Concentration (pmol gDW) + polymer		
Cytokinins	iPR	105.2 – 138.4	95-120		S
	iP	* 26.5 in one sample	21 – 32		
	cis Z	2.4 – 5.9	24 – 27		
	BA	1.8 – 2.3 x 10 ⁶	1.5 – 2.7 x 10 ⁶	_	
Auxins	IAA.	*804 in one sample	2.6 - 8.0 x 10 ³	Some effects after adding polymer	™ Ĥ N ⁶ - Benzyladenine
(A)	oxIAA	27 – 34 x 10 ³	22 – 28 x 10 ³		
Salicylic acid	SA	10 – 14 x 10 ³	4.6 – 6.6 x 10 ³		Kimitin Zzarin N ^a Kimiyikatin
			n = 4 ren	licates of fish	sludge Research

n = 4 replicates of fish sludge

Zhang et al. 2015, Talanta, with modifications Tarkowski and Yong (unpublished)











Partnership

Is Compost Tea GOOD Fertilizer?





Swedish University of Agricultural Sciences





Growing Together Gives More Rice & Food





Liu, Caspersen, Yong (2022) eLife







Insect (Black Solider Fly Larvae, BSFL) frass



Insect (Black Solider Fly Larvae, BSFL) frass







The Real World









FORMAS

Emerging market for Biostimulants

Why your garden needs GOGO Juice

Available at

POWERFEE

SUNNINGS

worehouse

Organic Amendments



Quality Control & Natural Product CONSISTENCY



NO.	PARAMETER	QUALITY STANDARD UNIT Based on Permentan No. RESULT 70/Permentan/SR.140/10/2011		
1.	Azotobacter	CFU/ml	≥ 10 ⁷	1,32 X 10 ⁷
2.	Pseudomonas	CFU/ml	≥ 10 ⁷	1,77 X 10 ⁸
3.	Lactobacillus	CFU/ml	≥ 10 ⁷	1,62 X 10 ⁸
4.	Saccharomyces	CFU/ml	≥ 10 ⁷	1,20 X 10 ⁸



1.	Nitrogen Fixing Activity	~	Positive	Positive
2.	Pathogenity Test		Negative	Negative
3.	рН	- H	3,0-8,0	7,6
4.	IAA	Mg/L		0,79
5.	Gibberelin	Mg/L	<u>6</u>	1,72
6.	Zaetin	Mg/L		1,23
7.	Kinetin	Mg/L		0,40

SUMMARY PRODUCT SPECIFICATION

1.	Escherichia coli	MPN/ml	< 10 ³	< 30
2.	Salmonella sp.	MPN/ml	< 10 ³	< 30

in accordance of Laboratory Tested BALITTANAH BOGOR West Java - Indonesia

Note :

* The Laboratory Testing by Balai Penelitian Tanah (Balittanah) Bogor, West Java - Indonesia.





Multi-functional Biofertilizer

2nd Prize

SEOUL INTERNATIONAL INVENTION FAIR – SIIF 2021, 1-4 DEC 2021







Summary





Eichmann et al. 2021



Coconuts = Earthworms = Birch sap







Kokosnötter = Daggmaskar = Björksav





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Biostimulant(s) signals influencing plant growth



New paradigm for **Plant Nutrition**



Wong et al. (2020) Wiley Wong et al. (2015) Springer Wong et al. (2016) Acta Horticulturae

What is a GOOD BioFertilizer? What is a HEALTHY Soil?

- Contains suitable mineral nutrients (NPK) (Macro - NPK & micro-nutrients as the building blocks)
- Contains natural growth promoting substances (Biostimulants)
- Improving soil structure & holds water
- Reduced incidence of plant diseases



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

- Marcel Proust



Thank You!

For more information,

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