Plant Biostimulants
Plant Regulators/PGRs
Plant Nutrients

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Biostimulants 2018

Biostimulants

Biostimulants are a new class of products for which legal definitions do not exist and regulatory pathways are unclear

	Fertilizers	Biostimulants	Pesticides
Description	• Provide macro and micro nutrients	• Improve nutrient use efficiency • Abiotic stress • Crop quality / Vigor	• Preventative or curative effects • Specific targets • PGR
Regulatory status	• Well known regulatory pathways • Low to medium level of requirements	• Regulatory pathways are unclear / under construction • Legal definitions are not established	• Well known regulatory pathways • High level of requirements

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Biostimulants 2019 – a Unique Category of Agricultural Input

Fertilizers

Substance containing one or more recognized plant nutrient(s) used for its plant nutrient content and designed for use or claimed to have value in promoting plant growth, except unmanipulated animal and vegetable manures, marl, lime, limestone, wood ashes and other products exempted by regulation

Macro secondary and micronutrients

DRAFT

DRAFT

Biostimulants

A substance or micro-organism, when applied to seeds, plants, or soil, supports or improves nutrient availability, uptake & efficiency, tolerance to abiotic stress or crop quality and yield. A plant biostimulant is neither a pesticide, plant regulator, fertilizer, agricultural liming material nor animal & vegetable manure

Microbial products, plant and algae extracts, humic acids, protein hydrolysates and amino acids, other

Pesticides

Prevent, destroy, repel or mitigate a pest or intended as a plant regulator, defoliant, or desiccant

Insecticide, fungicide, herbicide, plant regulator, defoliant, desiccant

Insecticides: carbamates, neonicotinoids, pyrethroids, microbials, etc.

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Biostimulant Definition

"A plant biostimulant is a substance(s), microorganism(s), or mixtures thereof, that, when applied to seeds, plants, the rhizosphere, soil or other growth media, act to support a plant's natural nutrition processes independently of the biostimulant's nutrient content. The plant biostimulant thereby improves nutrient availability, uptake or use efficiency, tolerance to abiotic stress, and consequent growth, development, quality or yield."

Definition History:

- First published in the U.S. in the USDA Report to Congress on Plant Biostimulants in December 2019
- December 2019 introduced to ISO and is being published in spring of 2022 as official ISO definition
- 2021 used in New Hampshire, Vermont (with small modification) and Delaware (agreed to use definition published by AAPFCO)
- February 2022 AAPFCO moved the definition to "tentative" status

Plant Biostimulant Categories

Plant Biostimulant Categories – from Guidelines for Verification of Plant Biostimulant Composition:

A. **Microbial Based Biostimulants**
   1. Live Microbial Products (e.g. *Rhizobacter sp., Bacillus sp., Azotobacter sp., Azospirillum sp., Glomus sp., Trichoderma sp.,* etc.)
   2. Complex Products Based on Non-Living Microorganisms and Their Metabolites

B. **Algal or Plant Extract Biostimulants**
   1. Aquatic Plant Extracts (e.g. derived from macroalgae such as *Ascophyllum sp., Ecklonia sp. Fucus sp., Kappaphycus sp., Laminaria sp., Sargassum sp., Ulva sp.,* etc.)
   2. Microalgal Extracts (e.g. derived from microalgae such as *Chlorella sp., Spirulina sp.,* etc.)
   3. Higher Plant Extracts (e.g. derived from plants such as *Allium sp., Brassica sp., Digitalis sp., Lupinus sp., Lycopersicon sp., Medicago sp.,* etc.)

C. **Complex Carbon-Based Biostimulants**
   1. Mined natural deposits (Humic substances) primarily composed of three fractions (humic acids, fulvic acids, and humin). Sources of humic substances are commercially harvested from terrestrial deposits which include, but are not limited to, leonardite, oxidized lignite, oxidized sub-bituminous coals, humalite, carbonaceous shales (including humic shale), peat, and sapropel.
   2. Other Complex Carbon-Based Residuals and Extracts (e.g. vermicompost/worm castings, compost waste materials, biochar etc.), or liquid extracts derived from these materials (e.g. compost tea, etc.).

D. **Protein hydrolysates with free amino acids Biostimulants - derived from plant, animal, or microbial proteins**
   1. Manufactured by chemical hydrolysis.
   2. Manufactured by enzymatic hydrolysis.

E. **Defined Molecules Purified from Minerals, Plants, Animals, Microbes, or obtained by Synthesis. These may include**:
   1. Organic molecules (e.g. amino acids, polyamines, polyphenols, betaines, oligosaccharides, alginates, carboxylic acids, fatty acids, chitin, chitosan etc.)
   2. Minerals not recognized as plant nutrients (e.g. silicon, selenium, etc.).

What do we know about Plant Biostimulants?

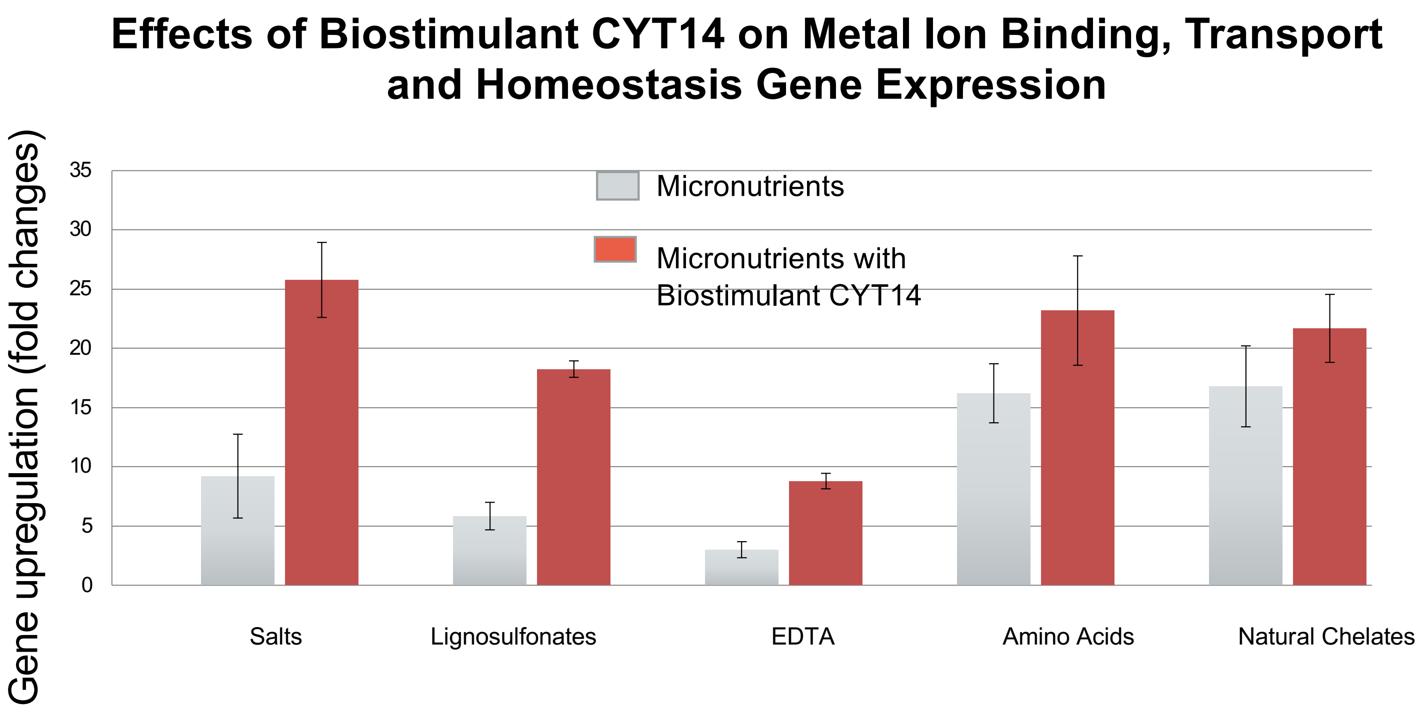
How do they compare to Plant Regulators/PGRs and Plant Nutrients?

Biostimulants and Nutrient Use Efficiency

molecular and whole plant level

Biostimulant and Plant Nutrients – molecular level

qPCR Results



- ✓ Nutrient solutions alone upregulated this gene involved in nutrient uptake
- ✓ CYT14 enhances expression of this gene over each nutrient solution alone
- ✓ Largest enhancement occurred with salts, lignosulfonates and EDTA
- ✓ **The nutrient is enhanced by the Biostimulant after 6 hours**

Canady et al. (2016)

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Biostimulant and Plant Nutrients – cellular level

ICP Results

Effects of Biostimulant CYT14 on Copper Concentration in the Tissue

Treatment	Salts (ppm)	Ligno-sulfonates (ppm)	EDTA (ppm)	Amino Acids (ppm)	Natural Chelates (ppm)
Micronutrients	16.7	16.9	16.0	18.5	22.94
Micronutrients with Biostimulant CYT13	20.7	23.0	23.0	27.3	25.0
Increase (%)	**24%**	**36%**	**42%**	**47%**	**9%**

CYT14 enhanced uptake of copper to the leaf tissue

Canady et al. (2016)

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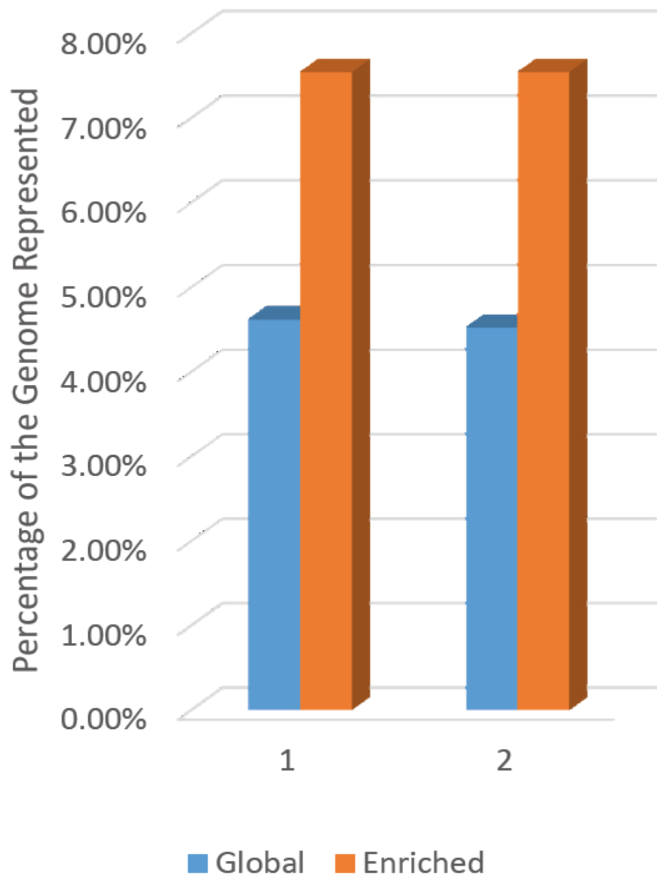
Biostimulant and Plant Nutrients – molecular level

Metabolite based Biostimulant effect on foliar dipotassium phosphate application (microarray results)



Application of Biostimulant with foliar dipotassium phosphate treatment enriched two gene groups involved in phosphorus metabolic processes

1. phosphorus metabolic process
2. phosphate-containing compound metabolic process

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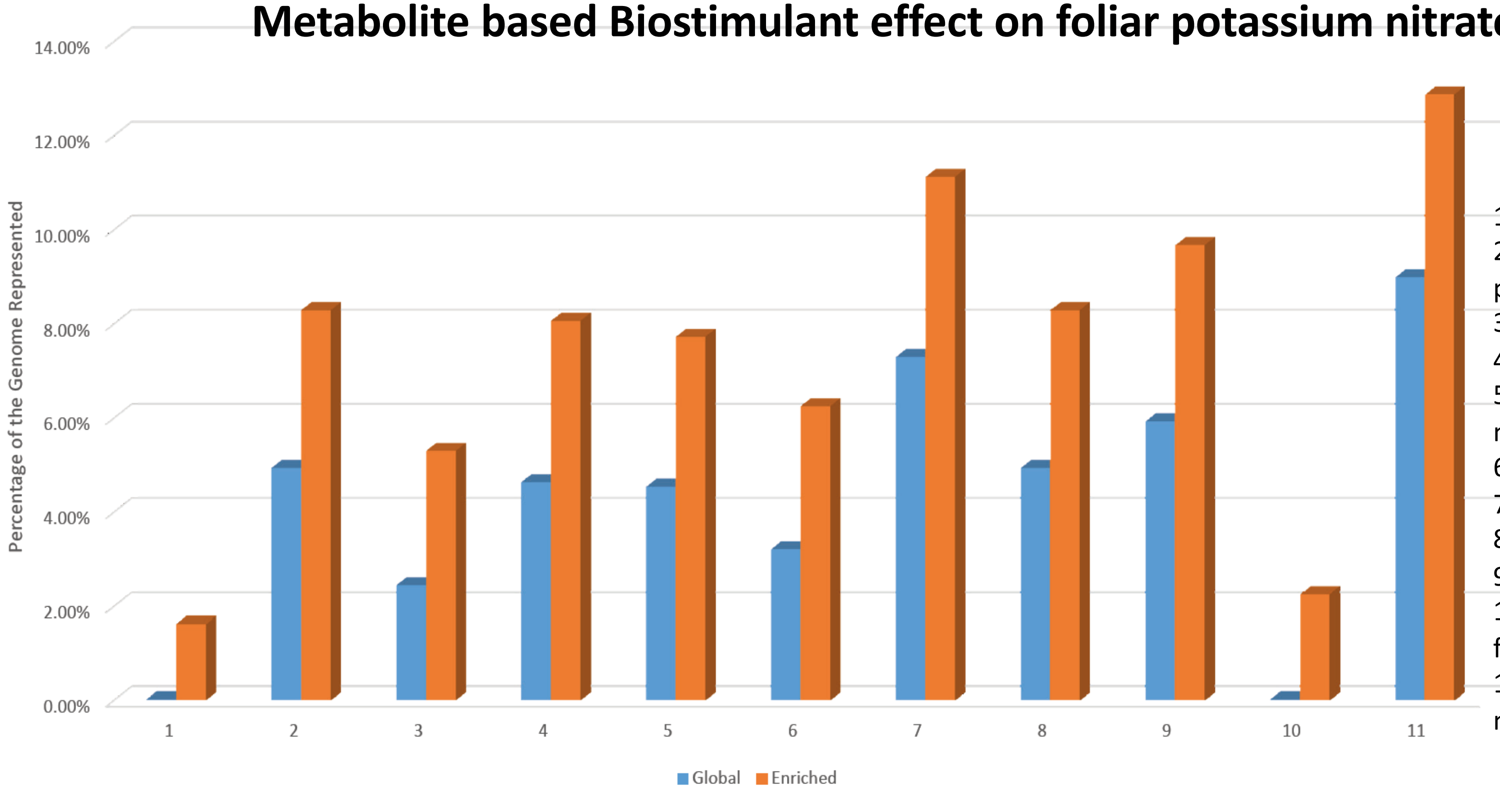
Biostimulant and Plant Nutrients – molecular level

Metabolite based Biostimulant effect on foliar potassium nitrate application



1. defense response to nematode
2. cellular protein modification process
3. protein phosphorylation
4. phosphorus metabolic process
5. phosphate-containing compound metabolic process
6. phosphorylation
7. protein metabolic process
8. protein modification process
9. macromolecule modification
10. regulation of defense response to fungus
11. organonitrogen compound metabolic process

Application of Biostimulant with foliar dipotassium phosphate treatment enriched eleven gene groups

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Biostimulant and Plant Nutrients – whole plant level

Effect of biostimulant application on nutrient uptake and distribution

Literature review:

43 different biostimulants were reported to improve nutrient use efficiency in 24 different crops

- Macro, secondary and micronutrients were evaluated
- Reported increased nutrient content in the tissue
- Reported reduction of fertilizer rate with comparable nutrient and/or yield results

Wozniak et al. (2020)

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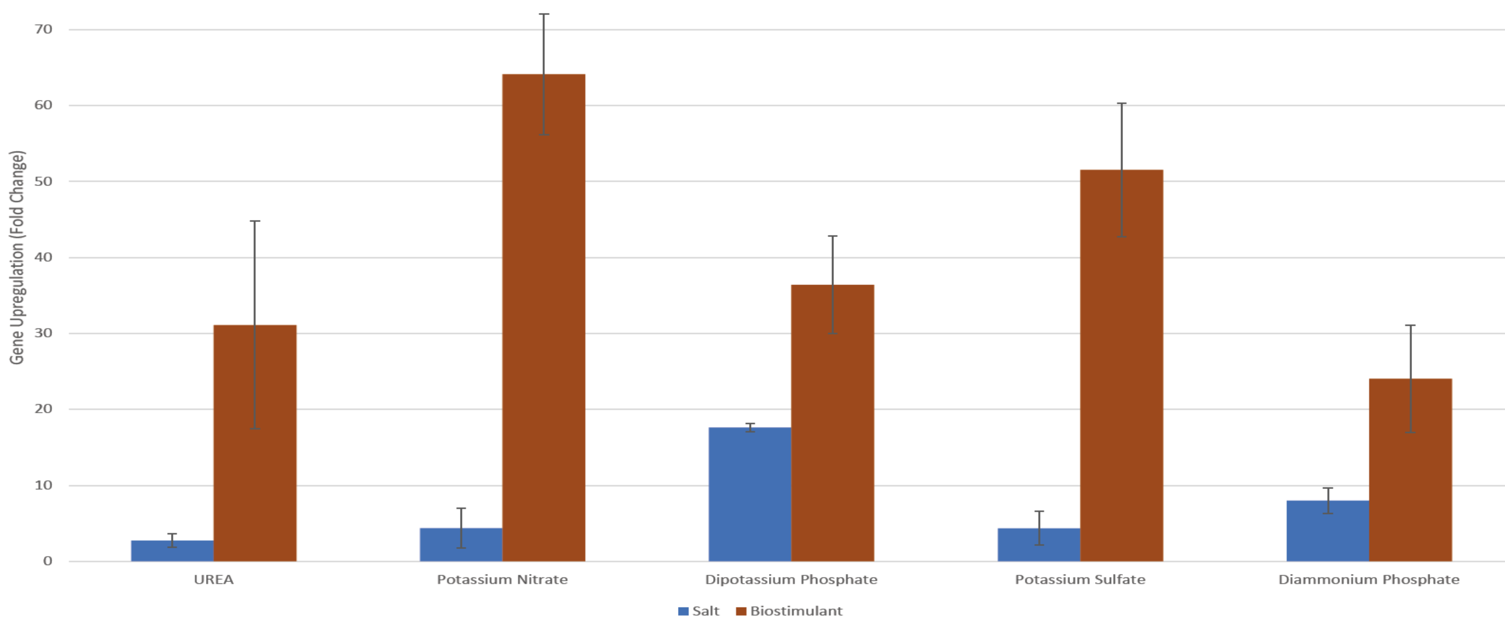
Biostimulants and Stress Tolerance

molecular and whole plant level

Biostimulant and Stress Tolerance - molecular level

Effect of foliar application of urea, potassium nitrate, dipotassium phosphate, potassium sulfate and diammonium phosphate with or without Biostimulant CYT726 on MSRB8 gene expression in *Arabidopsis thaliana* (qPCR results)



MSRB8 gene is involved in tolerance to oxidative stress

Schuster et al. (2018)

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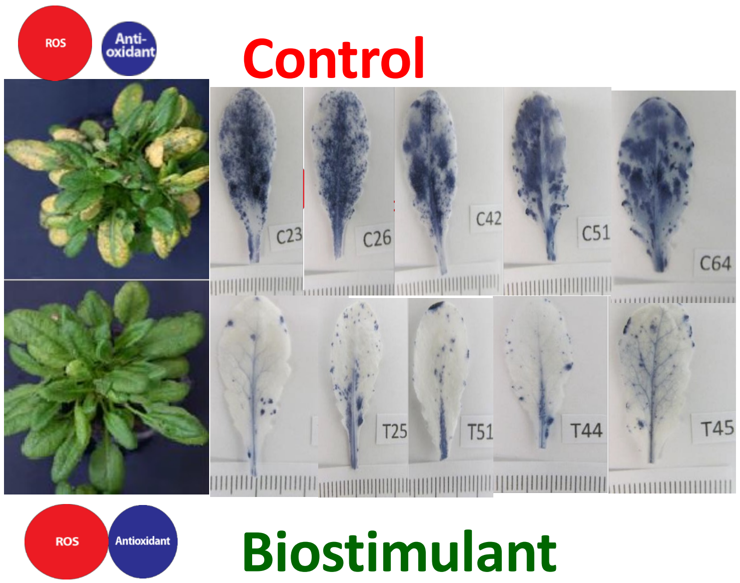
Biostimulant and Stress Tolerance - cellular and whole plant level



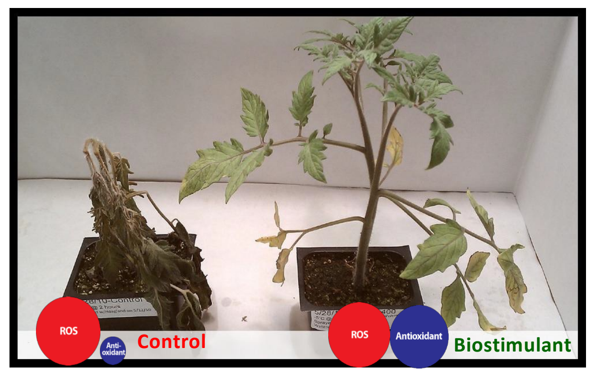
Leaves from *Arabidopsis* plants treated with Biostimulant showed much less ROS* production (less blue coloration) than not treated leaves

Treatment: application one hour before stress, then exposed to 39º F (4º C) cold stress for 1 hour Source: Cytozyme Laboratories Inc., USA

* ROS – Reactive Oxygen Species Blue coloration of superoxide radical with NBT solution..

Model plant: Tomato
Treatment: 2 weekly applications, then exposed to 23° F (-5° C) for 2 hours Source: Cytozyme Laboratories Inc., USA

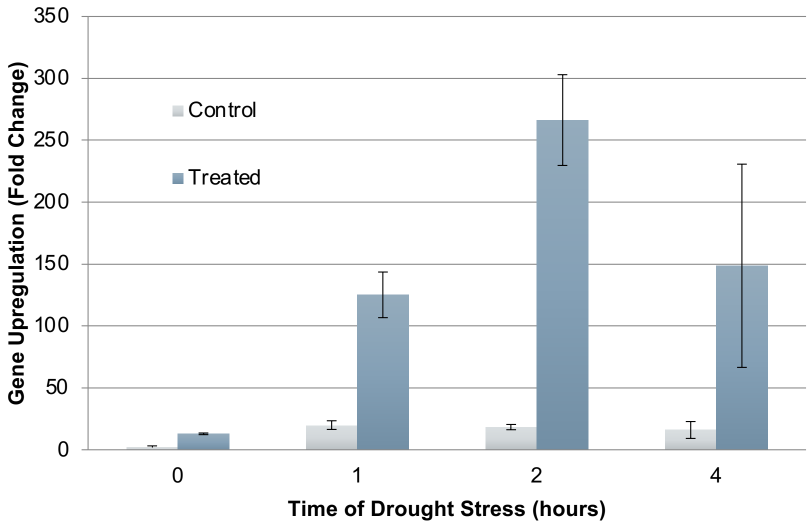
Tomato plants treated with Biostimulant showed better tolerance (no damage) to low temperature stress

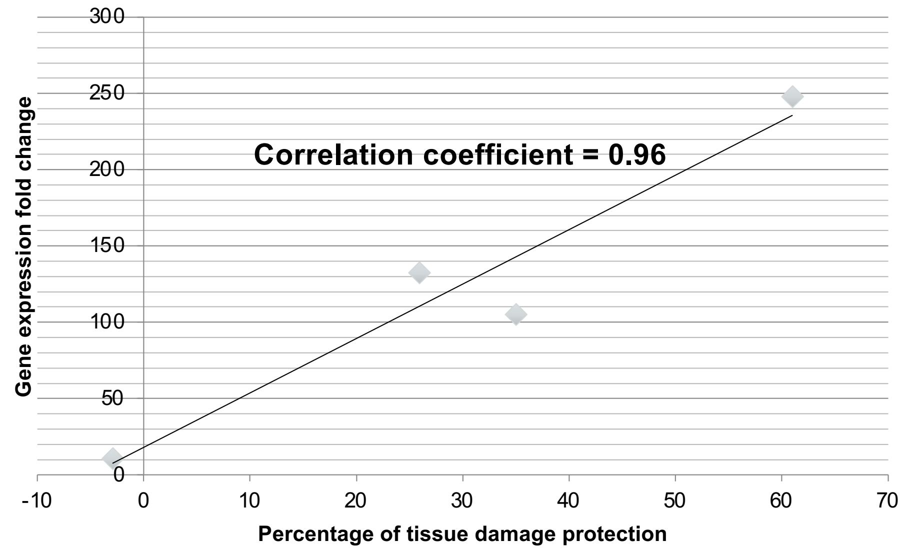


Biostimulant protected plants from overproduction of ROS* and damage caused by low temperature stress

Biostimulant and Stress Tolerance – molecular and cellular level

Biostimulant Upregulate Expression of Gene Involved in Drought Tolerance of Plants





- Biostimulant upregulated expression of drought tolerance gene 250-fold
- Tissue damage protection was highly correlated with drought tolerance gene

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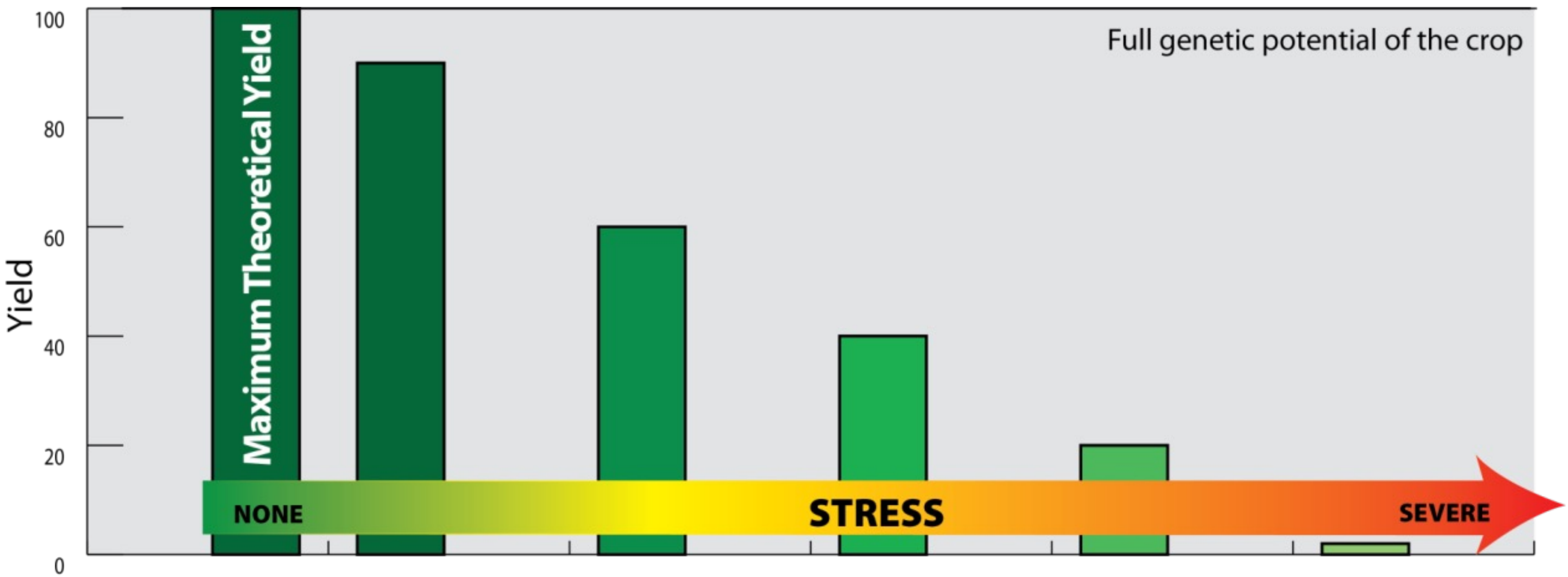
Blaszczak et al. (2016)

Biostimulants and Crop Yield

agricultural impact

Expressing Yield Potential with Biostimulants



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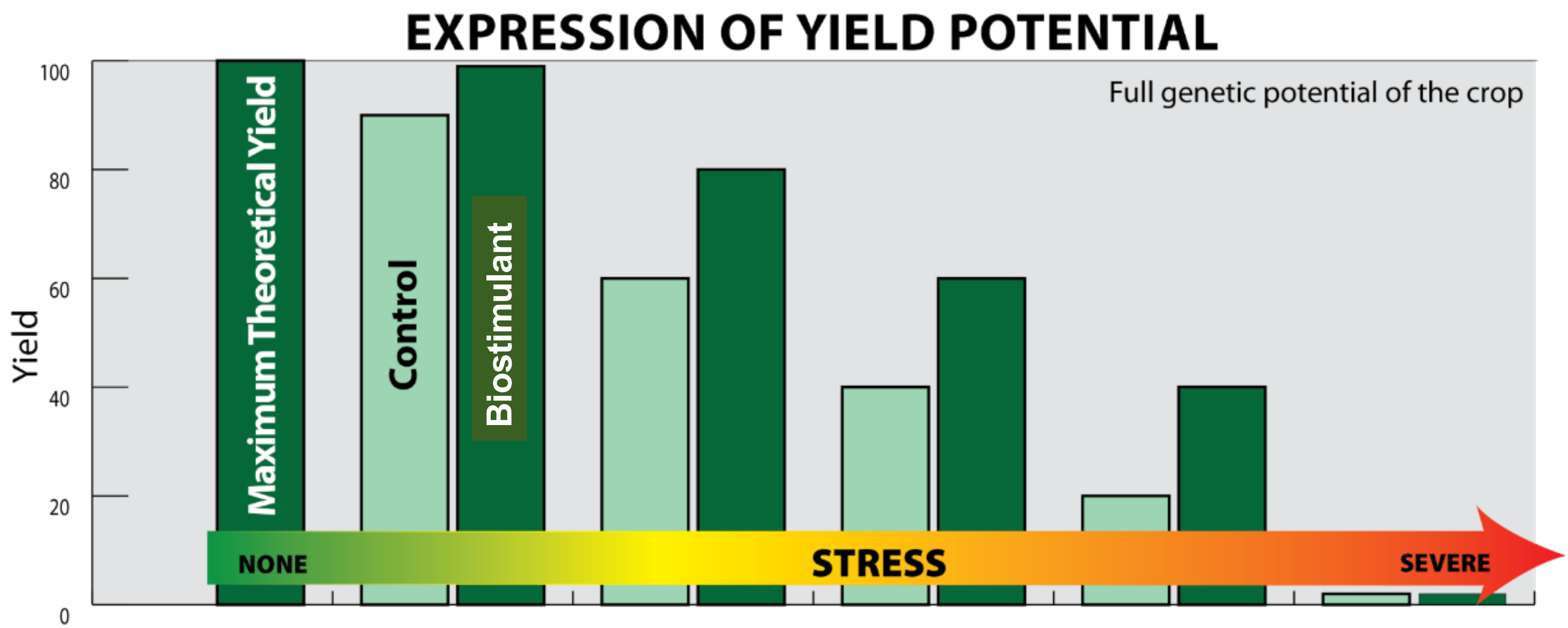
Expressing Yield Potential with Biostimulants



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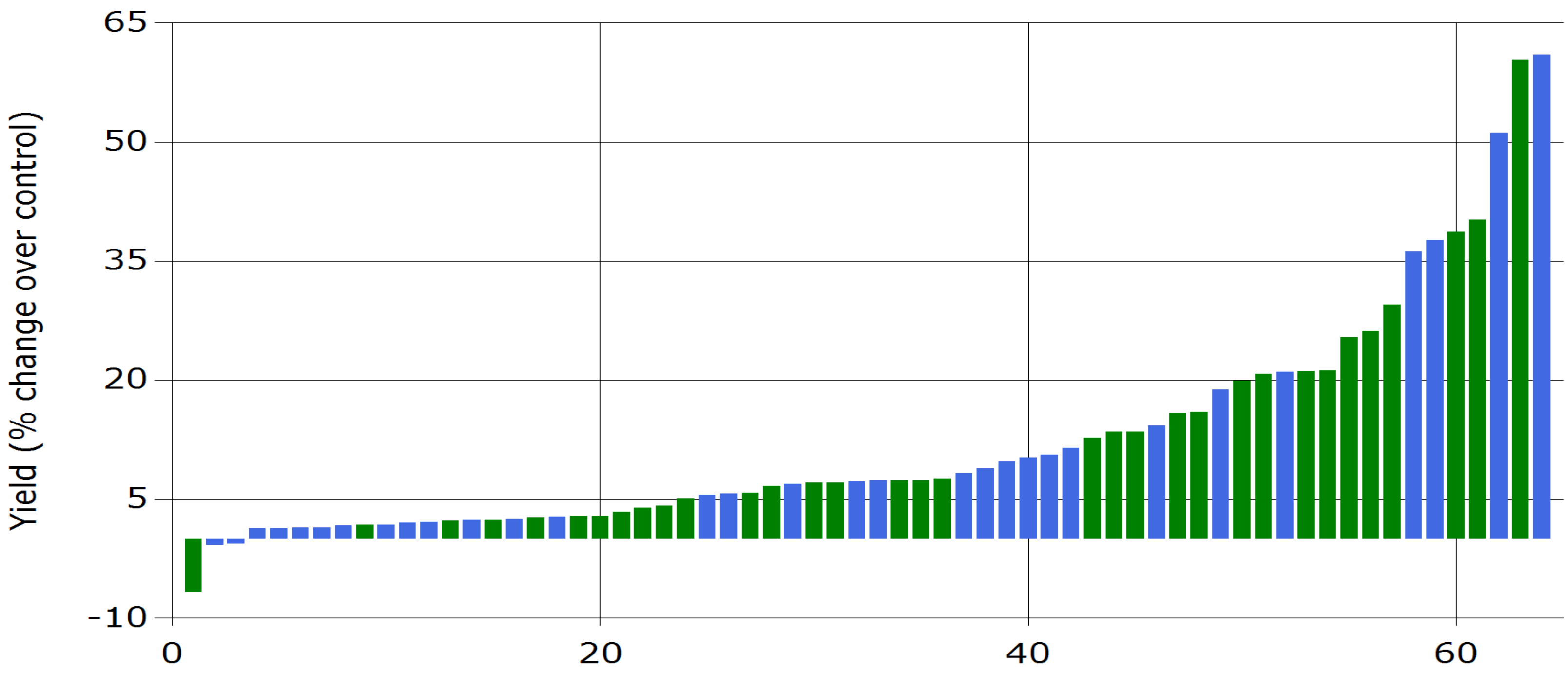
Expressing Yield Potential with Biostimulants



Biostimulant applied to granular fertilizer improved crop yield on average by 12.5% (n=64 in 4 countries)

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Blue color – 100% fertility level, green color – less than 100% fertility level for Biostimulant

Expressing Yield Potential with Biostimulants

Literature review: 15 groups of biostimulants, 126 trials, 380 treatments, over 70 crops tested

60% showed average 29% yield increase with $p \leq 0.05$
average 23% yield increase in agronomic crops
average 32% yield increase in horticultural crops

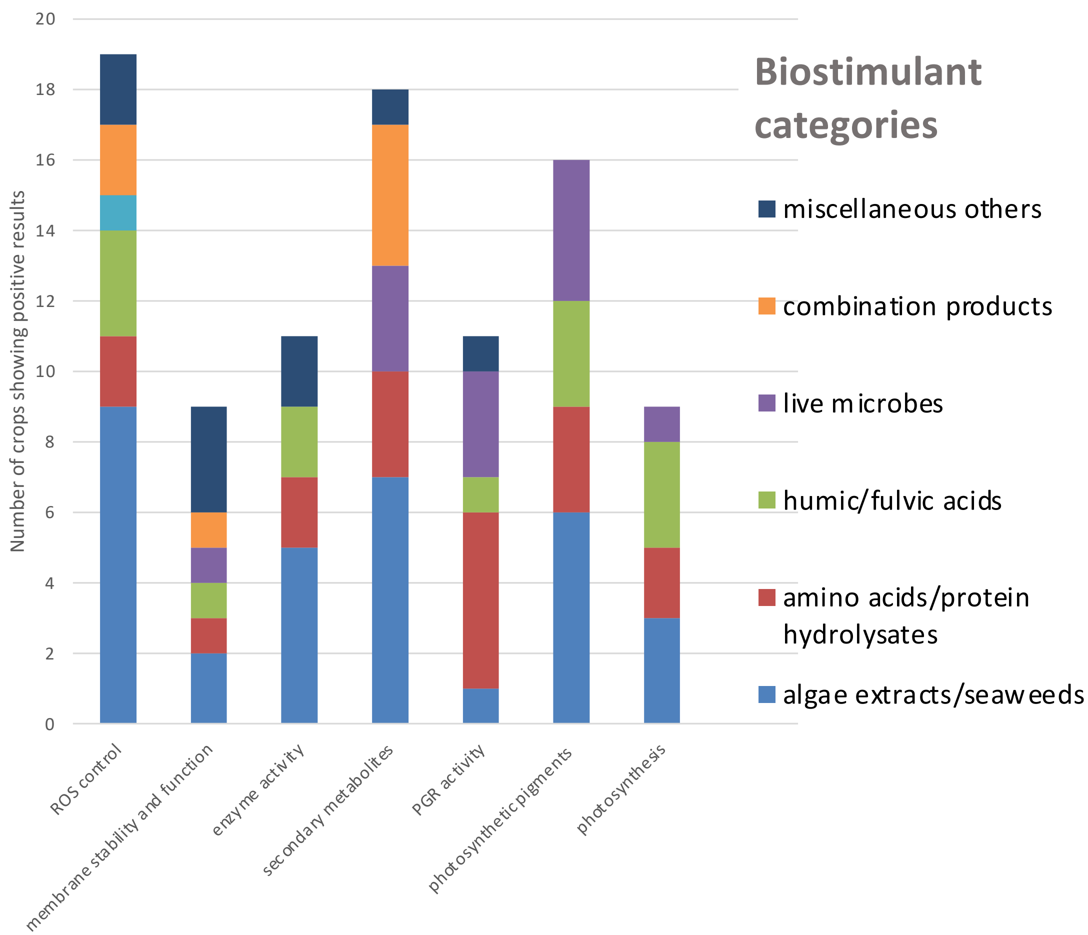
40% showed average 8% yield increase with $p \geq 0.05$

Wozniak et al. (2020)

Biostimulants Modes of Action

OVERVIEW

Biostimulant Modes of Action – cellular level OVERVIEW



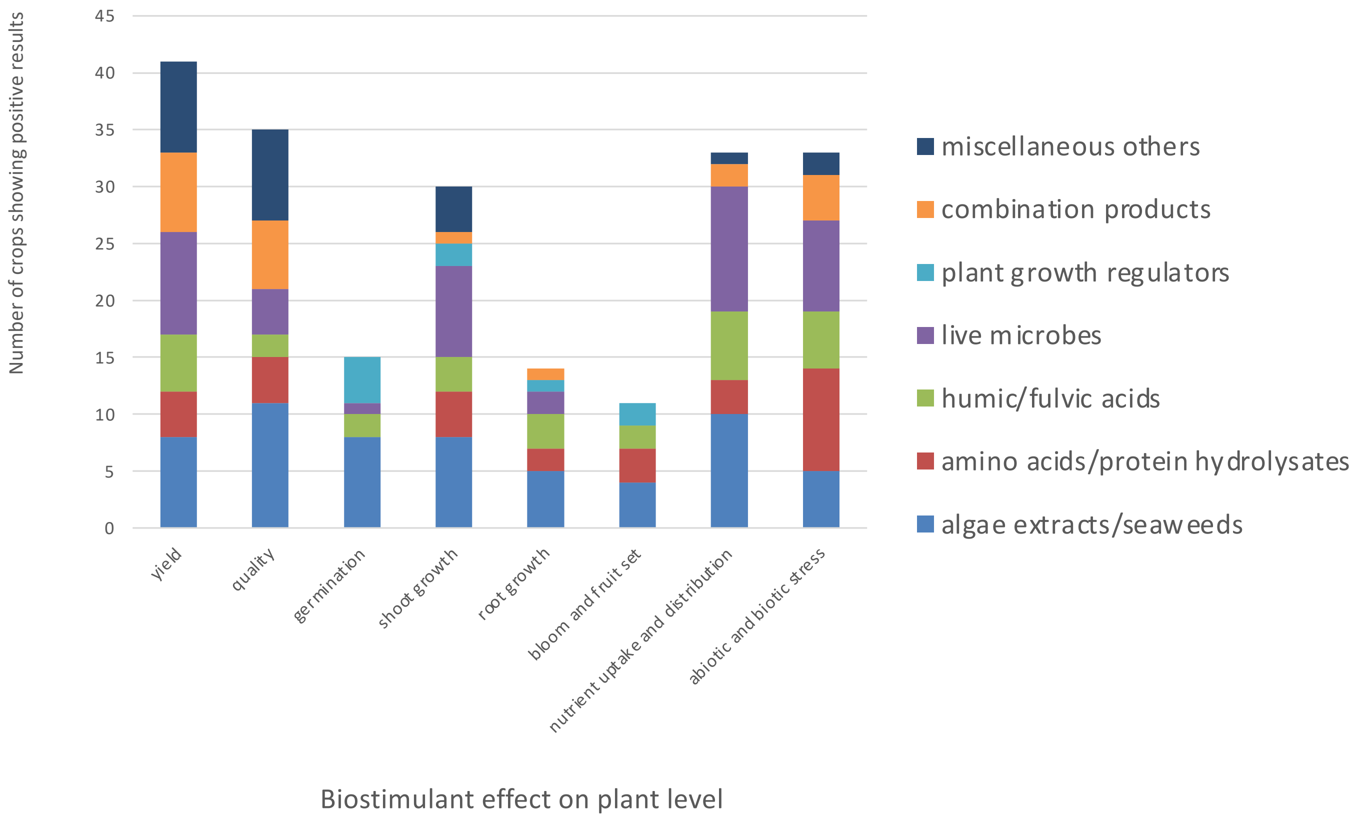
Biostimulant effect on cellular level

Number of crops showing positive response to biostimulant application. Six broad biostimulant categories were reported to improve reactive oxygen species (ROS) control, membrane stability and function, enzyme activity, secondary metabolite production, plant growth regulator (PGR) activity, photosynthetic pigment production and photosynthesis.

Over 50 biostimulants in over 30 crops

Wozniak et al. (2020)

Biostimulant Modes of Action – whole plant level OVERVIEW



Number of crops showing positive response to biostimulant application. Seven broad biostimulant categories were reported to increase yield, quality, germination, shoot growth, root growth, bloom and fruit set, nutrient uptake and distribution, and enhance crop performance under abiotic and biotic stress.

Over 80 biostimulants in over 60 crops

Wozniak et al. (2020)

Distinguishing Plant Biostimulants from Plant Nutrients and Plant Regulators/PGRs

PRESENTED EVIDENCE ON MOLECULAR, CELLULAR, WHOLE PLANT AND AGRICULTURAL LEVELS

"A plant biostimulant is a substance(s), microorganism(s), or mixtures thereof, that, when applied to seeds, plants, the rhizosphere, soil or other growth media, act to support a plant's natural nutrition processes independently of the biostimulant's nutrient content.

The plant biostimulant thereby

1) improves nutrient availability, uptake or use efficiency,
2) tolerance to abiotic stress,
3) and consequent growth, development, quality or yield."

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Thank You!

For Discussion

Differentiation between Biostimulants and Plant Regulators/PGRs

Biostimulants vs Plant Regulator/PGRs

- ✓ Complex biostimulants like seaweed extracts, humic acids, protein hydrolyzates with many mechanisms of actions – "limited support for hormonal basis biostimulation" *du Jardin et al. (2020), Stirk et al. (2020)*
- ✓ Antagonistic effects of PGRs *(Koprna et al. (2016)*
- ✓ Applied concentrations below active physiological levels *(Jithes et al. (2012), Stirk et al. (2014) and (2004)*
- ✓ Seaweed extract with removed gibberellins and auxins maintained its activity increasing maize biomass and grain yield *Mondal et al. (2015)*

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Biostimulants vs Plant Regulator/PGRs

- ✓ Natural *vs* synthetic
- ✓ Support *vs* disrupt homeostasis
  - ✓ Auxins in seaweed extracts *vs* as rooting agents for plant propagation (IBA, NAA, IAA) *vs* auxins as herbicides (2,4-D, MCPA, dicamba, picloram)
- ✓ Physiological vs excessive PGR concentrations
  - ✓ pmol/ml in seaweed extracts *vs* 25 ng/g DW in plant tissue compared to 40-50% in 2,4-D herbicide
- ✓ What methods to use for PGR testing?
  - ✓ Bioassays
  - ✓ Chemical analysis

Distinguishing Plant Biostimulants from Plant Regulators/PGRs and Plant Nutrients

Distinguishing Plant Biostimulants from Plant Regulators and Plant Nutrients

ESTABLISH WORKING GROUP

An objective of the US Biostimulant Industry is to present arguments for EPA to distinguish plant biostimulant products from plant regulators and plant nutrients and help EPA to more accurately interpret the definition of a plant regulator/plant growth regulator using current scientific knowledge.

To develop US Biostimulant Industry's *technical* position on differentiation of products to be registered as plant regulators/plant growth regulators from biostimulants and plant nutrients taking into consideration plant physiological responses at molecular, cellular, whole plant and agricultural levels.

This technical position must be developed with academic support to validate (add credibility to) the findings to be presented to EPA.

Distinguishing Plant Biostimulants from Plant Regulators and Plant Nutrients

Literature search guidelines and deliverables.
For every effect and product category from the table below the following information must be collected:

- List of author(s) and publication year
- Abstract
- Link to whole publication and PDF of the publication
- Evaluation of publication quality (peer-review article, poster, extension publication, etc.) – for further discussion in the established working group
- Data retrieval:
  - Product category, name, and composition, if applicable
  - Application rate(s)
  - Application method (soil, seed, foliar, etc.) and timing
  - Plant/crop used in the research
  - Effect measurement(s) with statistical analysis (p-value indication if listed) – to be further defined by the established working group
  - Agronomic/economic benefit if measured (better seed germination, shoot and root growth, seedling growth, plant establishments, plant/crop size/mass/flowering, fruit set, maturation and consequently yield and quality, etc.)
- Author's conclusions on the effect (one sentence) indicating why it supports or does not support specific claim
- Mode of action, if applicable, with indication if measured or hypothesized

Product effect	PRODUCT CATEGORY How does each category impact specified effect?		
	FERTILIZERS	BIOSTIMULANTS	PLANT GROWTH REGULATORS
1. Nutritional support claims and consequent agronomic/economic importance			
Enhanced nutrient availability through mobilization, chelation/complexation, solubilization			
Enhanced nutrient uptake			
Enhanced nutrient assimilation			
Enhanced nutrient retention			
Enhance nutrient utilization by plant/crop			
Reduction of disorders caused by nutrient deficiency			
Reduction of nutrient loss to the environment			
Reduction of fertilizer application rates			
Modes of action: *Gene regulation influencing or influenced by nutrient management in the plant*			
Others			
1. Abiotic stress tolerance or resistance claims and consequent agronomic/economic importance			
Tolerance to drought			
Tolerance to salinity			
Tolerance to high/low temperature			
Tolerance to agrochemical overapplication			
Tolerance to other abiotic stress			
Crop recovery after stress			
Modes of action: *Gene regulation influencing plant tolerance to abiotic stress; ROS neutralization; enzymatic activity; membrane stability; photosynthesis*			
1. Influence of each product category on Plant Growth Regulators metabolism, homeostasis, and action			
PGR claims: -natural vs synthetic PGRs -physiological concentrations vs overload -support vs disrupts homeostasis -bioassay vs chemical analysis			

Distinguishing Plant Biostimulants from Plant Regulators and Plant Nutrients

ADDITIONAL TOPICS FOR EVALUATION – FROM bpia MEETING

Carbon sequestration
Reduction of gas emission
Environmental stewardship
Soil health
Others

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Please join this exciting literature search opportunity to benefit the agriculture and sustainable food production worldwide

contact BPIA leaders or
elizabeth.wozniak@vlsci.com

Thank You!