Plant Protection and Plant Health in Europe

Efficacy and risks of „biorationals“ in organic and integrated pest management - acceptable?

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Microorganisms between Plant Protection and Biostimulation

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Focus of the Talk:

Vital Microbial Inoculants Applied in Agricultural Practice

Plant beneficial bacteria and fungi

Definition Biostimulant:

Plant biostimulants are any (substances or) microorganisms applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content.

Plant biostimulants stimulate natural processes *in planta*!

Biostimulation Features

- Growth promotion, incl. root growth (lateral root development)

  Enhanced nutrition efficiency

  Hormonal effects, hormone-like (bioactive) compounds
- Abiotic stress tolerance

  (drought/flood, salt/osmotic, heat/cold, heavy metal, photo, oxidative)
- Enhanced crop resilience/vigor
- Early flowering, enhanced flowering
- Crop quality maximization (value-adding ingredients)
- Post-harvest traits (extended shelf-life, decay delay, foraging capacity)

Definition Biocontrol Agents

The control of one organism by another.
Biocontrol agents are living (micro)organisms
protecting plants against their enemies, i.e. reducing
the population of pests or diseases to acceptable levels.

Biocontrol Features

- Antibiosis – anti-microbial metabolites (antagonism)
- Competition – for space (occupying ecological niches)
  for macro/micro nutrients
  for Iron (chelators, siderophores)
- Parasitism – myco-parasitism (antagonism)
  bacterial (*Actinomycetes*)
  fungal (*Trichoderma* spp.)
- Induced Systemic Resistance (ISR) – cell walls (callose, lignin, phenolics)
  enzymes (chitinase, peroxidase, polyphenoloxidase, PAL)
  small molecules (phytoalexines)

Conflicting Contents

Biostimulation

- Abiotic stress tolerance (mediated by plant signaling cascades)

  via perception of bioactive substances

  induction/release of signaling compounds

Biocontrol

- Induced Systemic Resistance (mediated by plant signaling cascades)

  via perception of bioactive substances

  induction/release of signaling compounds

Overlap

Term: **Broad-spectrum resistance**
pathogens, insects and/or nematodes but also against abiotic stresses

- Multiple-stress responsive genes
- Shared responses in major biotic and abiotic stress pathways
- Biotic and abiotic crosstalk in several gene networks

ELSEVIER

Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks

Miki Fujita[1,2,3], Yasunari Fujita[4], Yoshiteru Noutoshi[5,6], Fuminori Takahashi[1,3,7], Yoshihiro Narusaka[8], Kazuko Yamaguchi-Shinozaki[2,4,9] and Kazuo Shinozaki[1,2,3]

Plants have evolved a wide range of mechanisms to cope with biotic and abiotic stresses. To date, the molecular mechanisms that are involved in each stress has been revealed comparatively independently, and so our understanding of convergence points between biotic and abiotic stress signaling pathways remain rudimentary. However, recent studies have revealed several molecules, including transcription factors and kinases, as promising candidates for common players that are involved in crosstalk between stress signaling pathways. Emerging evidence suggests that hormone signaling pathways regulated by abscisic acid, salicylic acid, jasmonic acid and ethylene, as well as ROS signaling pathways, play key roles in the crosstalk between biotic and abiotic stress signaling.

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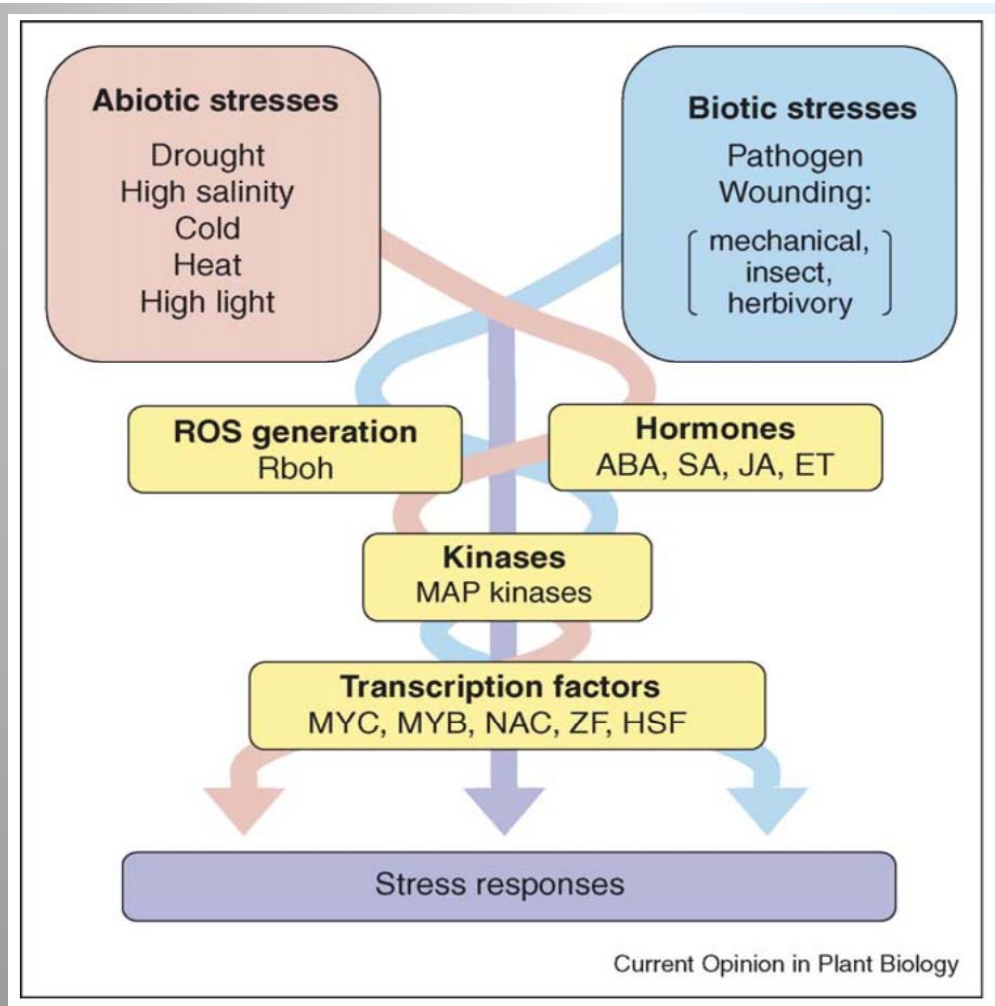
Introduction

Plants undergo continuous exposure to various biotic and abiotic stresses in their natural environment. To survive under such conditions, plants have evolved intricate mechanisms to perceive external signals, allowing optimal response to environmental conditions. Phytohormones such as salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA) are endogenous, low-molecular-weight molecules that primarily regulate the protective responses of plants against both biotic and abiotic stresses via synergistic and antagonistic actions, which are referred to as signaling crosstalk [1,2,3••]. Moreover, the generation of reactive oxygen species (ROS) has been proposed as a key process that is shared between biotic and abiotic stress responses [4,5•]. Rapidly accumulating data, resulting from large-scale transcrip-

Plant signaling pathways consist of complex networks with frequent crosstalk, allowing plants to regulate both abiotic stress tolerance AND disease resistance.



Convergence points in abiotic and biotic stress signaling networks.

Convergence Points

Abscisic Acid (**ABA**) – abiotic stress response
Jasmonic Acid (**JA**), Salicylic Acid (**SA**), Ethylene (**ET**), Hypersensitive Reaction (**HR**)
Reactive Oxigen Species (**ROS**) – biotic stress response

Respiratory burst oxidase homolog (*rboh*), required for ROS production
induces ABA and HR → crosstalk point

Enhanced **r**esponse to **A**BA 3 (*era3*) allelic to **e**thylene **in**sensitive 2 (*ein2*)
intersection in ABA and ET signaling → crosstalk point

Jasmonate **in**sensitive 1 (*jin1*) allelic to MYC2 transcription factor (tf)
Low MYC2 enhances JA/ET pathogen responses → crosstalk point

MYB2 (tf) highly homolog to *Botrytis* susceptible 1 (*bos1*), mediated by JA
bos1 mediates biotic and abiotic stress signaling via ROS → crosstalk point

NAC RD26 (tf) induced by JA, H_2O_2, pathogens, also by drought, salt, ABA
functions in pathogen defense, senescence, ABA signaling → crosstalk point

Convergence Points

MAPK (mitogen-activated protein kinase) cascades include multiple stress responses

MKK2 – MPK4/6 cascade involved in cold and salt stress signaling

MKK4/5 – MPK3/6 cascade regulates pathogen defense via WRKY22/29 (tf)

MPK3/6 also induced by abiotic stress (via ABA) → crosstalk point

MPK6 – induces ET production via ACC (aminocyclopropan 1 carbocylic acid) synthase

induces JA-dependent root growth and MYC2 expression → crosstalk point

activates ROS signaling via serine/threonine kinases → crosstalk point

inducible by H_2O_2 and ABA → crosstalk point

*Os*MPK5 ortholog to *At*MPK3, induced by ABA and infection in rice → crosstalk point

the plant journal

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MAPK kinase 10.2 promotes disease resistance and drought tolerance by activating different MAPKs in rice

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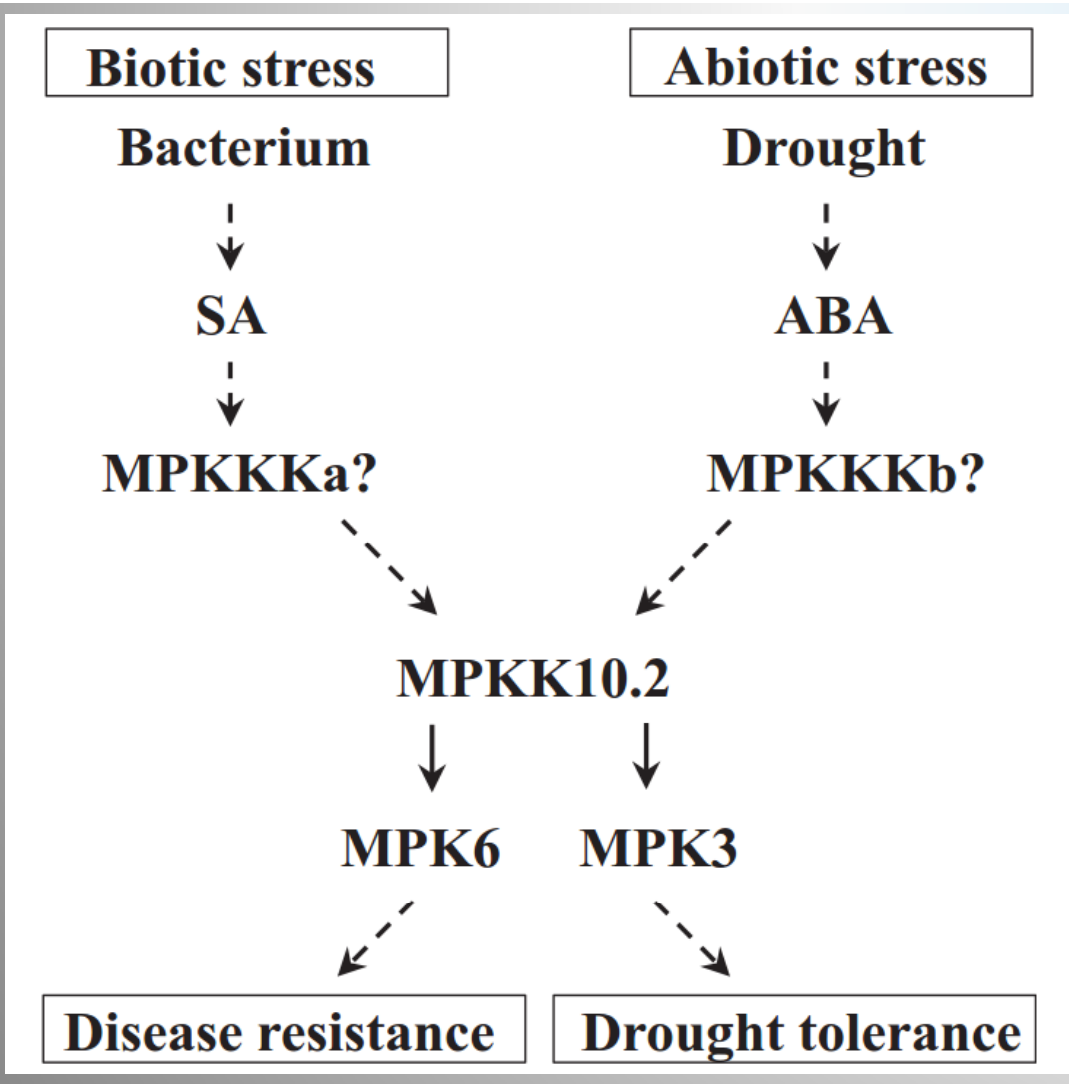
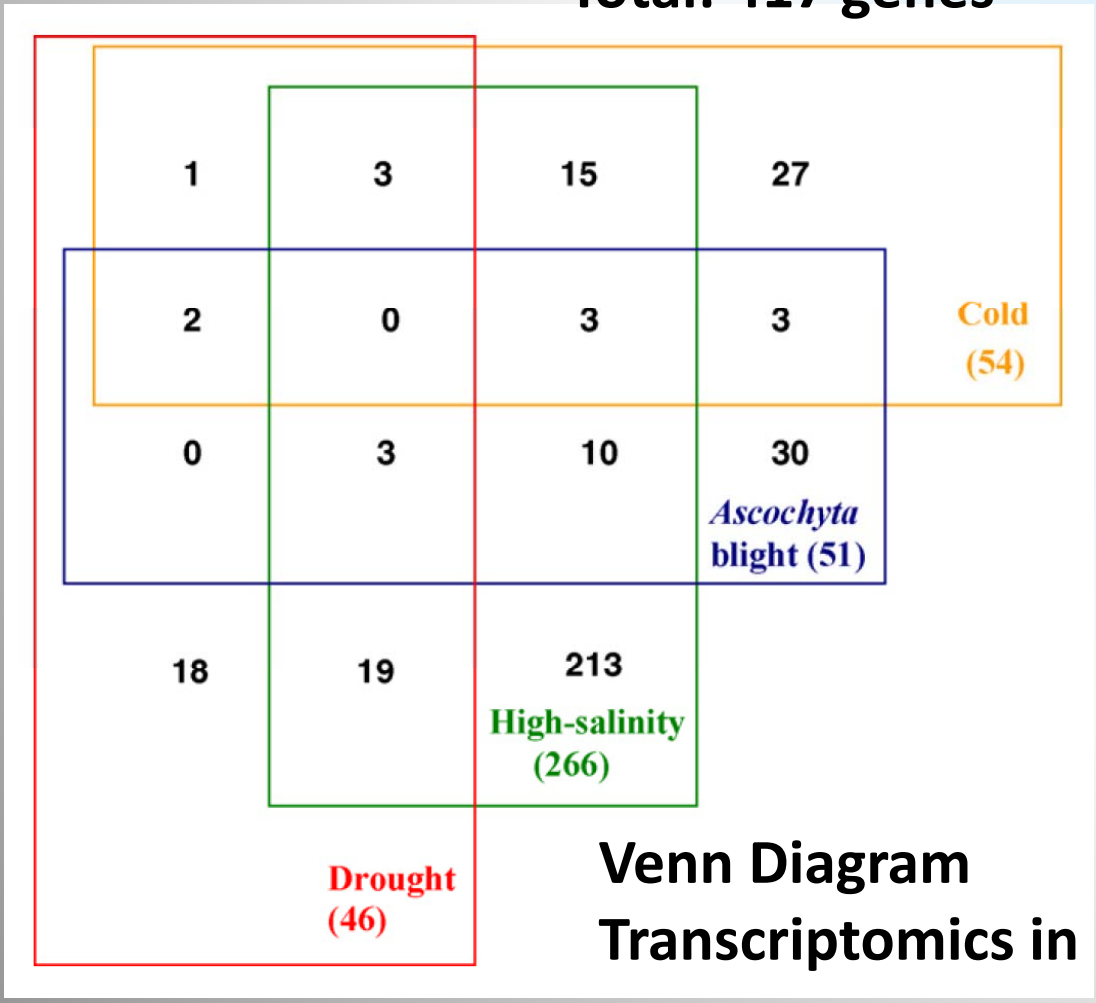
SUMMARY

Mitogen-activated protein kinase (MAPK) cascades, with each cascade consisting of a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAPK, have important roles in different biological processes. However, the signal transduction in rice MAPK cascades remains to be elucidated. We show that the structural non-canonical MAPKK, MPKK10.2, enhances rice resistance to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), which causes bacterial streak disease, and increases rice tolerance to drought stress by phosphorylating and activating two MAPKs, MPK6 and MPK3, respectively. *MPKK10.2*-overexpressing (oe) plants showed enhanced resistance to both *Xoc* and drought, whereas *MPKK10.2*-RNA interference (RNAi) plants had increased sensitivity to both *Xoc* and drought. MPKK10.2 physically interacted with MPK6 and MPK3, and phosphorylated the two MAPKs *in vivo*. Transcriptionally modulating *MPKK10.2* influenced MPK6 phosphorylation during rice-*Xoc* interaction, and *MPKK10.2*-oe/*MPK6*-RNAi double mutants showed increased sensitivity to *Xoc*. *MPKK10.2*-oe/*MPK3*-RNAi double mutants showed survival rates similar to those of control plants, although the survival rates of *MPKK10.2* transgenic plants changed after drought stress. These results suggest that MPKK10.2 is a node involved in rice response to biotic and abiotic responses by functioning in the cross-point of two MAPK cascades leading to *Xoc* resistance and drought tolerance.

Keywords: MAPK, bacterial streak, drought, phosphorylation, *Oryza sativa*.

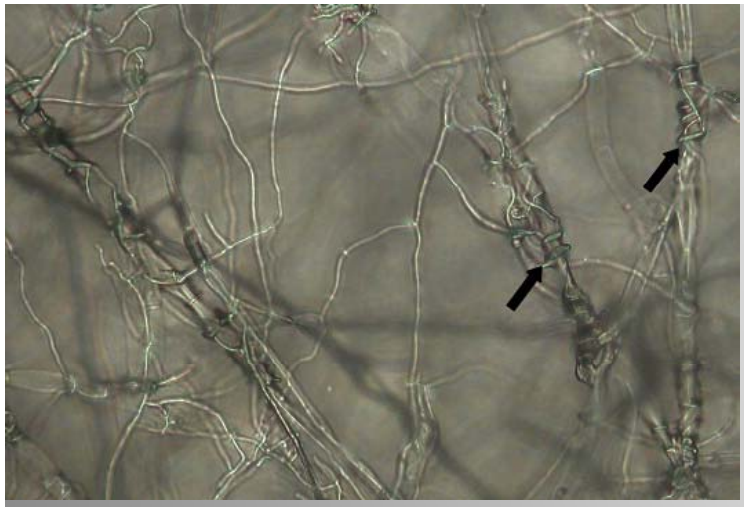

Biotic stress
Abiotic stress
Bacterium
Drought
SA
ABA
MPKKKa?
MPKKKb?
MPKK10.2
MPK6
MPK3
Disease resistance
Drought tolerance

Total: 417 genes

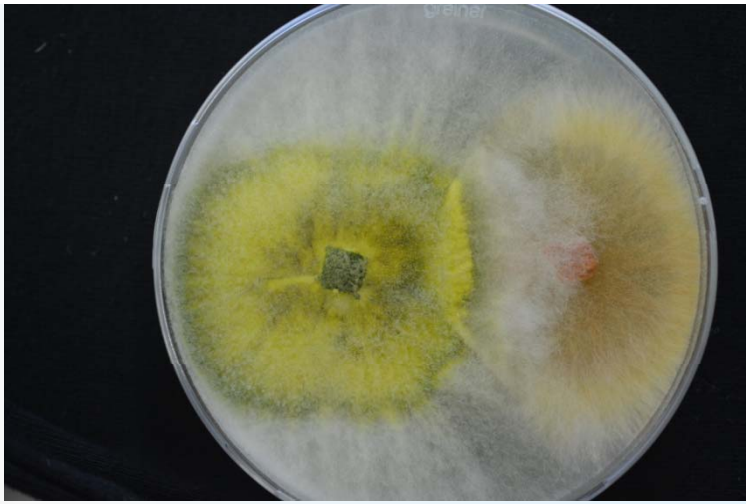


Venn Diagram
Transcriptomics in *Cicer arietinum*

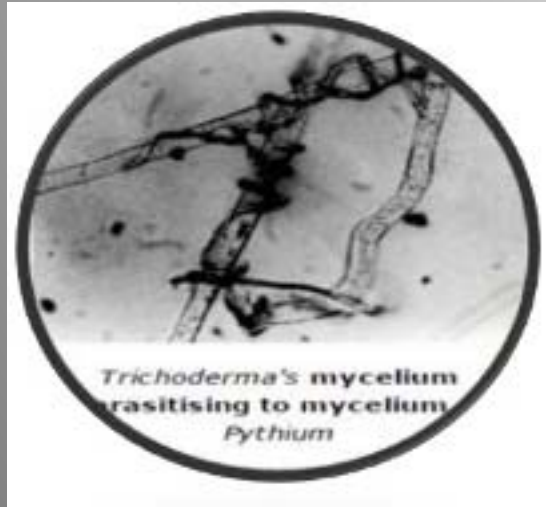
Why should microbial biostimulants
that activate multiple biotic and abiotic stress pathways
for enhanced broad-spectrum crop resistance
be classified as (bio)pesticides ?

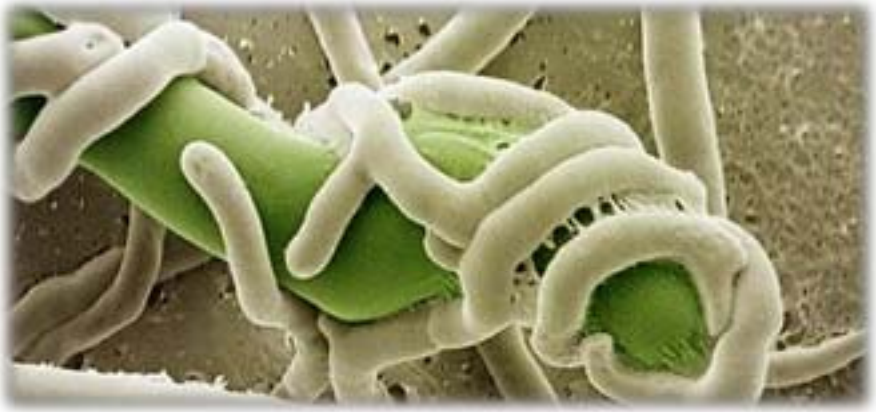


Biocontrol



What we got…..





Bacterial Biocontrol Agents

Genera: *Bacillus*, *Pseudomonas*, *Burkholderia*, *Serratia*, *Streptomyces*, *Actinoplanes*, *Stenotrophomonas*, *Paenibacillus*, *Klebsiella*, *Alcaligenes*, *Pantoea*, *Ralstonia*…

Competition for nutrients and space (root niches, root adhesion)
motility, chemotaxis, detox. antimicrobial root compounds
root exudates and mucilage (sugars, organic acids, amino acids)

Allelopathy/Allelochemicals:
Iron-chelating siderophores, antibiotics, biocidal volatiles, antifungals

Antibiosis: amphisin, oomycin, phenanzine, oligomycin, zwittermicin, tensin…..
Lytic enzymes, cell wall hydrolases

Detox/degradation: antibiotics, phytotoxine produced by pathogens

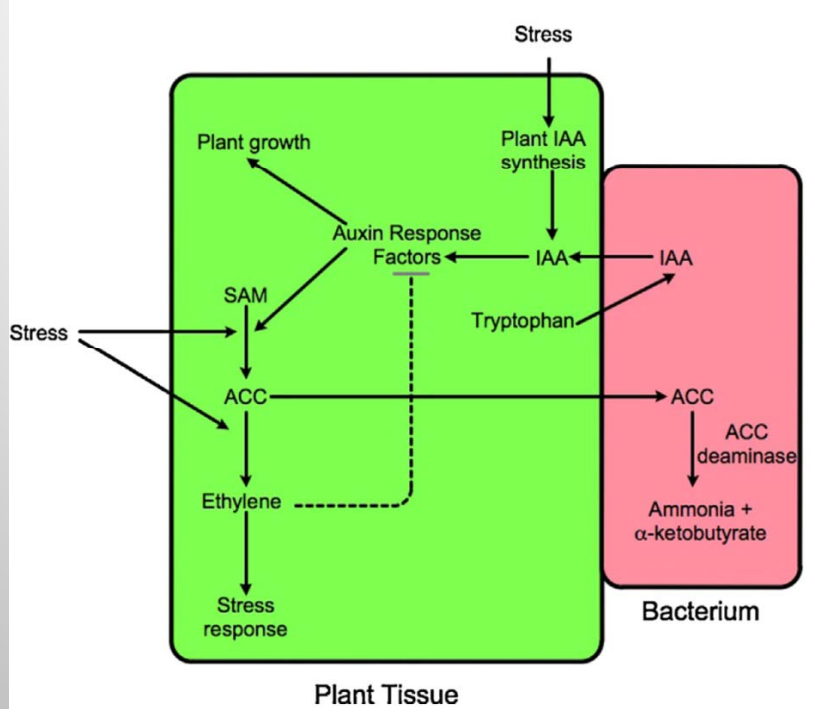
ISR/Priming: flagellin, siderophores, lipopolysaccharide, volatiles
induce plant defense chemicals (callose, phenolics, lignin, phytoalexins,
lytic enzymes, PR-proteins)

Bacteria for Biostimulation

Genera: *Rhizobium*, *Pseudomonas*, *Bacillus*, *Paenibacillus*, *Bredybacillus*, *Rhanella*, *Azotobacter, Azospirillum*, *Klebsiella*, *Acinetobacter*, *Stenotrophomonas*, *Serratia…*

Growth promo: N_2-fixation, nutrient solubilization (P), auxin and IAA production, ACC deaminase production, ammonia, HCN, siderophores, mycorrhiza helper functions

Abiotic stress tolerance:
Bacterial auxin, IAA and SA production
Decreased heavy metal toxicity
Micro-nutrient mobilization



Fungal Biocontrol Agents

Genera: *Trichoderma*, *Talaromyces*, *Cladorrhinum*, *Idriella*, *Penicillium*, *Chaetomium*, *Minimedusa*, *Coniothyrium*…
avirulent *Fusarium*, *Rhizoctonia* and *Phialophora* strains

Competition for nutrients and space (infection sites)

Antibiosis: Gliovirin, Gliotoxin, Glucose-Oxidase (H_2O_2 production)

Mycoparasites: *Trichoderma*, *Leucosporidiella*, *Platygloea*, *Rhodotorula*, *Sporidiobolus*

ISR: avirulent strains, *Trichoderma*, *Penicillium*

Effector molecules (elicitors): xylanase, oligogalacturonide, chitosan

Fungi for Biostimulation

Genera: *Trichoderma*, *Glomus* (AMF), endo/ekto-mycorrhizal fungi, endophytes...

Abiotic stress tolerance:

Sebacina, *Piriformospora*, *Neotyphodium*, *Trichoderma*, *Curvularia, Acremonium*

mainly salt, drought and cold stress

- triggering stress response systems in plants
- produce anti-stress chemicals (osmolyte, loline, sugar/alcohols)

oxidative stress: ROS / RNS detox., antioxidants

Plant growth promotion:

Neotyphodium positively influences photosynthetic CO_2 fixing in host plants

Piriformospora produces cytokinine

Paecilomyces produces IAA and gibberelline

Overlaps again

Bacteria in biocontrol and biostimulation
Bacillus, *Pseudomonas*, *Paenibacillus*, *Klebsiella*, *Serratia*, *Stenotrophomonas*

Fungi in biocontrol and biostimulation
Trichoderma, *Penicillium* – mycorrhiza and endophytes

Should competition really be a biocontrol trait ?

Regulation, Legislation

Microorganisms should be identified at the strain level (**not always easily done!**) postulating that most biological activities are strain-specific (**right or wrong?**)

Biostimulants

should be defined according to the **intended agricultural outputs**
nutrient efficiency may cover nutrient mobilization, uptake, transport, storage, assimilation but also root development
abiotic stress tolerance physical or chemical stressors of non-biological origin
quality traits may be very diverse (from nutritional value to shelf-life)

Registration is possible
under national fertilizer legislations **or**
EU PPP regulation

Why not register under the EU fertilizer laws ?

Impossible, BIOSTIMULANTS cannot be fertilizers under EU legislation !
Here, fertilizers are clearly defined:

- Provide nutrients for plants
- **Primary nutrients**: NPK
- **Secondary nutrients**: Ca, Mg, Na, S
- **Micro-nutrients**: boron, cobalt, copper, iron, manganese, molybdenum, zinc
  excl. inorganic materials (exception: chelating or complexing agents)

All **microorganisms that interact with plant physiology** must be registered under the PPP regulation, even if they do not protect plants from pests or diseases.

Subject:	Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL laying down rules on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009

(4) Decision No 768/2008/EC of the European Parliament and of the Council[4] lays down common principles and reference provisions intended to apply across sectoral legislation in order to provide a coherent basis for revision or recasts of that legislation. Regulation (EC) No 2003/2003 should therefore be replaced by a Regulation drafted to the extent possible in accordance with that Decision.

(5) Contrary to most other product harmonisation measures in Union legislation, Regulation (EC) No 2003/2003 does not prevent non-harmonised fertilisers from being made available on the internal market in accordance with national law and the general free movement rules of the Treaty. In view of the very local nature of certain product markets, this possibility should remain. Compliance with harmonised rules should therefore remain optional, and should be required only for products, intended to provide plants with nutrient or improve plants' nutrition efficiency, which are CE marked when made available on the market. This Regulation should therefore not apply to products which are not CE marked when made available on the market.

Currently no harmonized frameworks are existing neither in the EU, nor in the US

EC regulation No 1107/2009 on plant protection products ('PPPs') is applicable to all categories of biostimulants and biocontrol agents, considering a very broad definition of PPPs.

Lengthy and costly procedure to place a PPP on the European market.

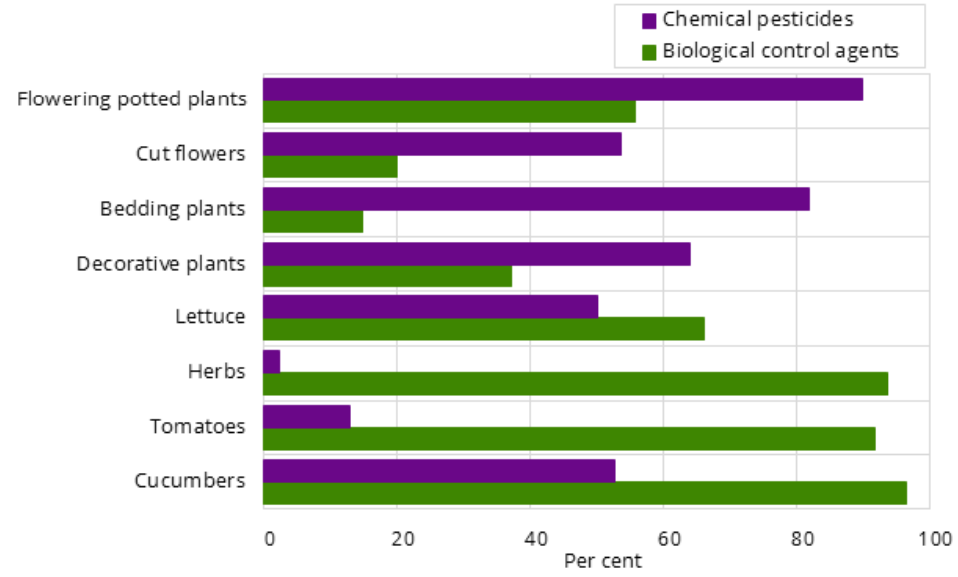
Alternative: The national fertilizer legislations !

BCA Market

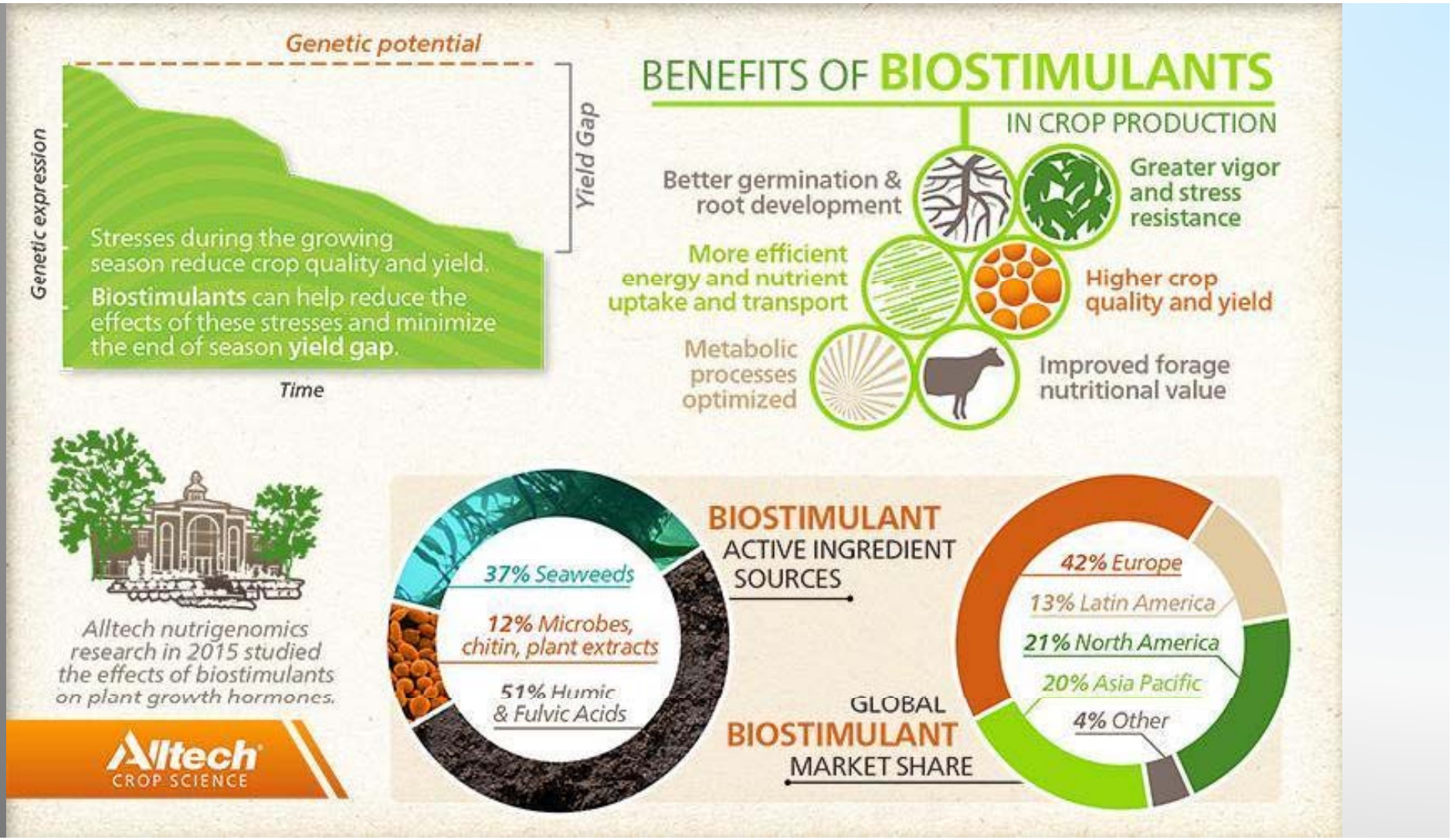
Top Players

BASF
Syngenta
Bayer CropScience Biologics
DuPont
Novozymes
Koppert Biological Systems
Monsanto Company
Marrone Bio Innovations
Biobest
Certis USA
Andermatt Biocontrol

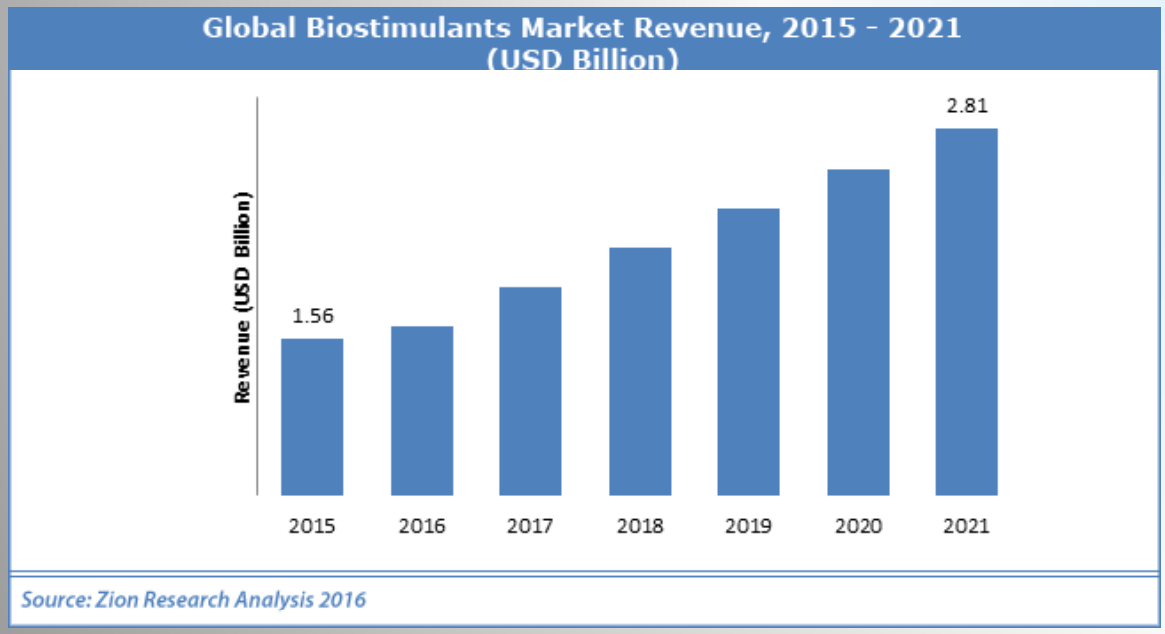
Figure 1. Share of greenhouse area treated[1] with biological control agents and chemical pesticides in 2015



The BCA market is expected to reach 3 billion USD by 2020 (1.7 b. USD in 2015)
growth rate > 10%, approx. 4% of the global pest control market


Genetic potential
Genetic expression
Yield Gap
Stresses during the growing season reduce crop quality and yield.
Biostimulants can help reduce the effects of these stresses and minimize the end of season yield gap.
Time
BENEFITS OF BIOSTIMULANTS
IN CROP PRODUCTION
Better germination & root development
Greater vigor and stress resistance
More efficient energy and nutrient uptake and transport
Higher crop quality and yield
Metabolic processes optimized
Improved forage nutritional value
Alltech nutrigenomics research in 2015 studied the effects of biostimulants on plant growth hormones.
Alltech CROP SCIENCE
37% Seaweeds
12% Microbes, chitin, plant extracts
51% Humic & Fulvic Acids
BIOSTIMULANT ACTIVE INGREDIENT SOURCES
GLOBAL BIOSTIMULANT MARKET SHARE
42% Europe
13% Latin America
21% North America
20% Asia Pacific
4% Other

Comparable to BCA market: 1.7 to 3.0 billion USD



Due to the **lack of acceptance** of biostimulant concepts, market data are of limited reliability. The **regulatory status** of biostimulants is diverse, depending on registration as fertilizer or pesticide, authorized or not in organic production. Biostimulants may be spread over many regulations, without being named as such.

Conclusions

- **Overlaps exist** between Biostimulation and Biocontrol
  biotic/abiotic stress responses, ISR
  microbial genera/species/strains applied
- **Traditional definition** of Biocontrol is narrow and precise (**implies direct interaction**)
  definition for regulation/registration is very broad
  mainly because indirect effects – **mediated by the crop** – were included
  indirect effects **better apply to biostimulation** – stimulate natural processes *in planta*
- **Harmonized frameworks** for differentiation, regulation and registration **are lacking**
  forcing SMEs to **register biostimulants under PPP** would outcompete most companies
  only multinational enterprises could afford millions USD per registered product
  loophole escapes using national laws are preferable
  current EU proposal on fertilizers opens this route **on purpose (?)**
- **Markets are fast growing** – all could participate (as long as PPP is avoided ☺)
  broad participation serves the good aim of “sustainable agriculture”

Latest Trends in Biostimulation and Biocontrol

- Strain combinations
- Complex microbial consortia

Mixtures are considered to be more effective than single strains!

- Synergistic effects
- Enhanced rhizosphere competence (mixtures of fungi and bacteria)

Route of EU regulation for **microbial combination products** not yet determined

Examples exist for substances:

- UVCB (unknown/variable composition biological) substances (EU-REACH regulation)
- Botanicals (one or more components found in plants) under EU PPP regulation

REACH: registration, evaluation, authorization of chemicals

CRISPR-Cas

Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats - **C**RISPR-**as**sociated nucleases

Genome editing occurs as a natural process
Programmable nucleases induce specific double-strand breaks and the cell's own mechanisms repair the breaks by natural processes.

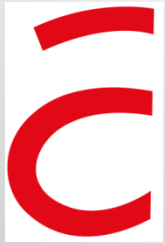
GMO: Involves promotors (35S), transgenes and selection markers (kanamycin)

CRISPR-Cas: possible solely on the protein level (guide-RNAs and/or DNA Oligos)
Leaves no footprints in the edited genomes – CRISPR testing difficult

Discussion still pending – will CRISPR be classified as GMO technique or not?

CRISPR-strains: **to see, or not to see?** – That is the question!

Thank You !



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