

# Innovative Tools to Understand the Mode of Action of Biostimulants

An online course by New Ag International,  
a division of Informa Connect

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# Lesson Overview

Part 1: How to investigate the mode of action of biostimulant products

Part 2: New technologies to support the market positioning of biostimulant products

Part 3: The importance of partnerships: the BIOSTIMOLA project and public-private collaboration

# Part 1: How to investigate the mode of action of biostimulant products

- EU Green Deal
- Function defines the product
- Mechanism and mode of action
- Analytical determinations
- Real Time PCR Gene expression
- Transcriptome analysis (RNA seq)



# EU Green Deal



## 2030 Targets for sustainable food production

### PESTICIDES



Reduce the overall use and risk of chemical and hazardous pesticides

### NUTRIENT LOSSES



Reduce nutrient losses by 50% whilst retaining soil fertility, resulting in 20% less fertilisers

### ANTIMICROBIALS



Reduce sales of antimicrobials for farmed animals and aquaculture

### ORGANIC FARMING



Increase the percentage of organically farmed land in the EU

#EUFarm2Fork

#EUGreenDeal



# Function defines the products



## Issues



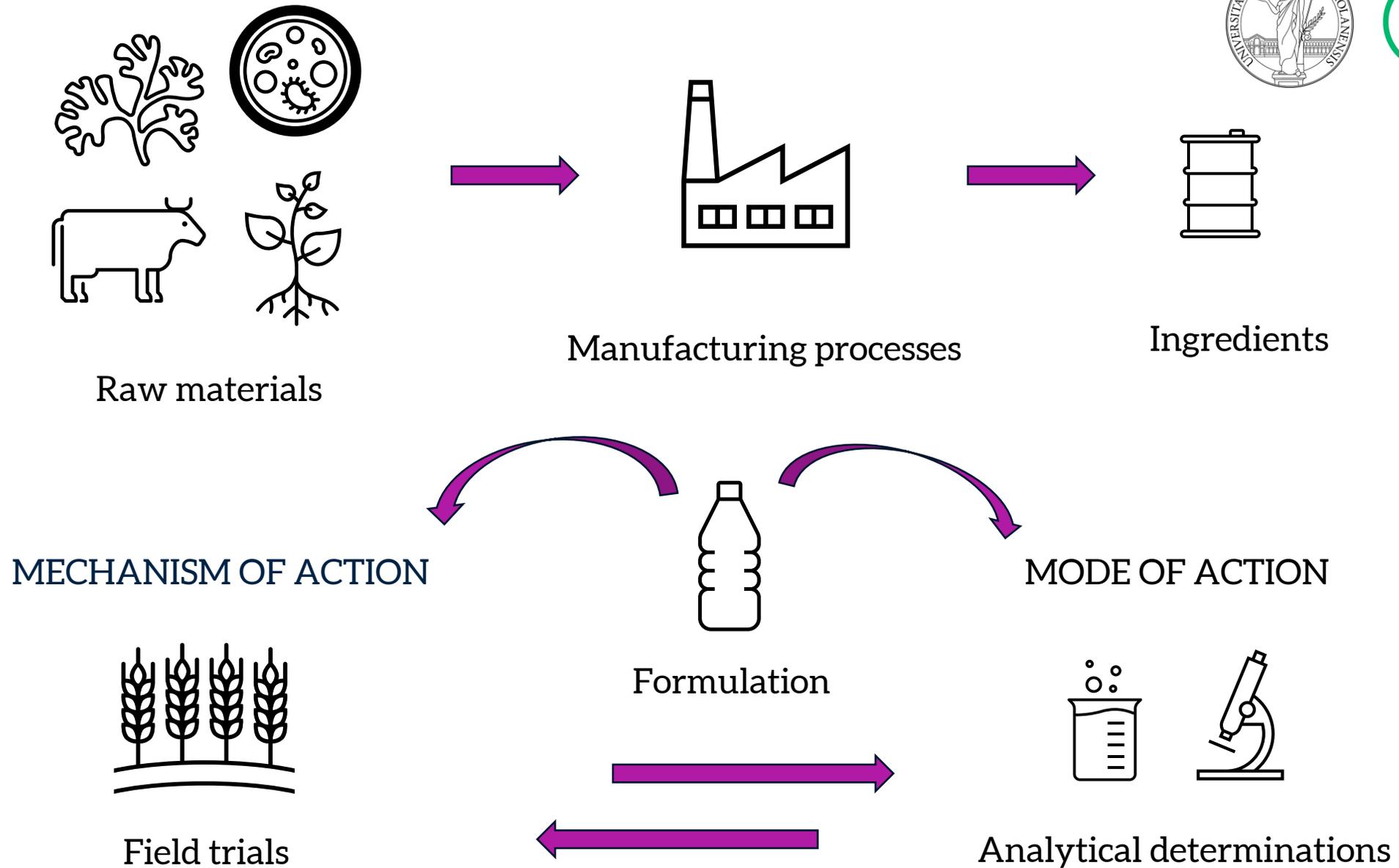
Function defines the product.



All plant biostimulants need Single Market access.



Plant biostimulants contribute to climate-smart agriculture



# Mechanism and mode of action



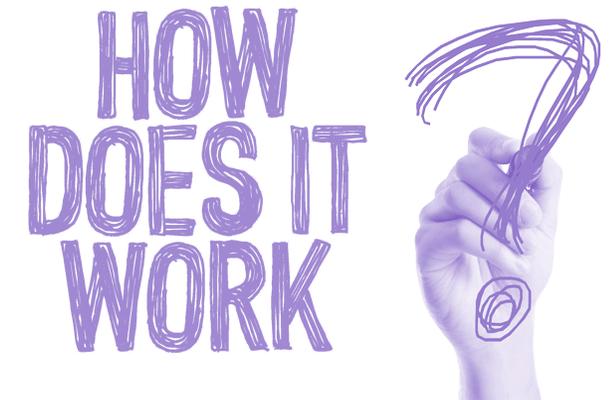
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## MECHANISM

It consists of identifying a tangible effect of the biostimulant action (e.g., a stimulation of photosynthesis or yield) without an understanding of the explicit biochemical or molecular "mode of action".

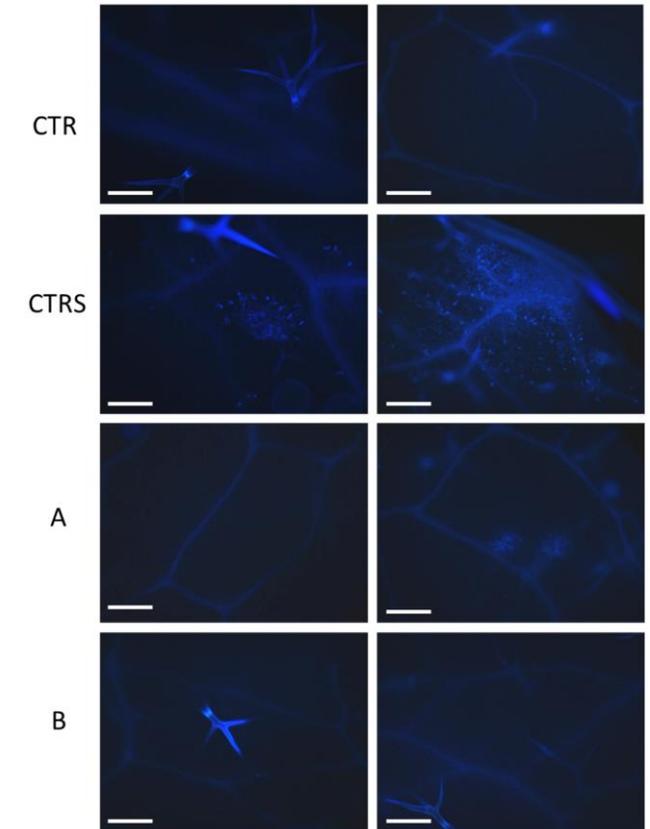
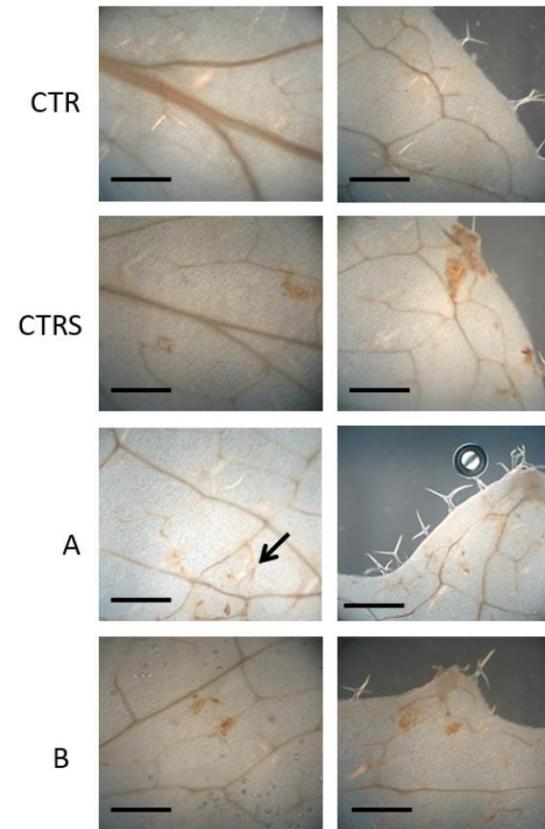
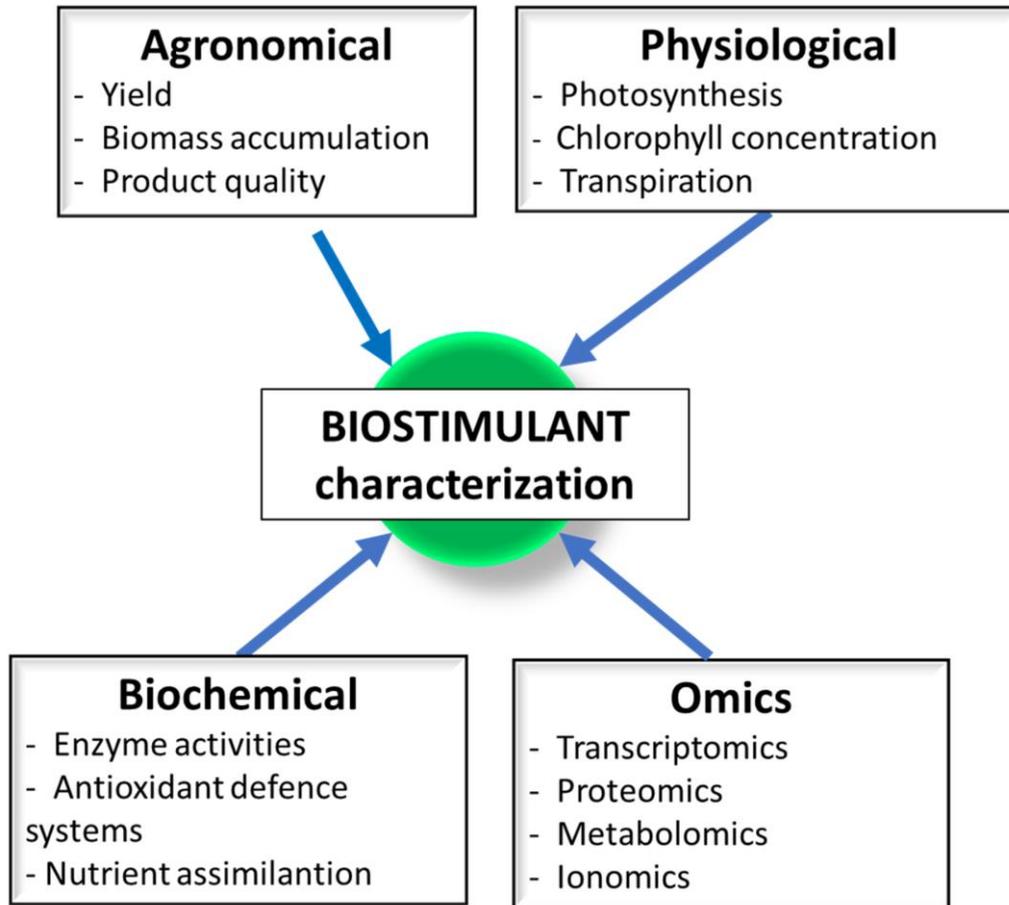
## MODE OF ACTION

It involves the identification of a biochemical, molecular or physiological process.



**If we accept the concept that a biostimulant is a product of clear benefit but unknown mode of action, then it can only be regulated by its safety and proof of efficacy.**

Yakhin, O. I., Lubyantsev, A. A., Yakhin, I. A., & Brown, P. H. (2017). Biostimulants in plant science: a global perspective. *Frontiers in plant science*, 7, 2049



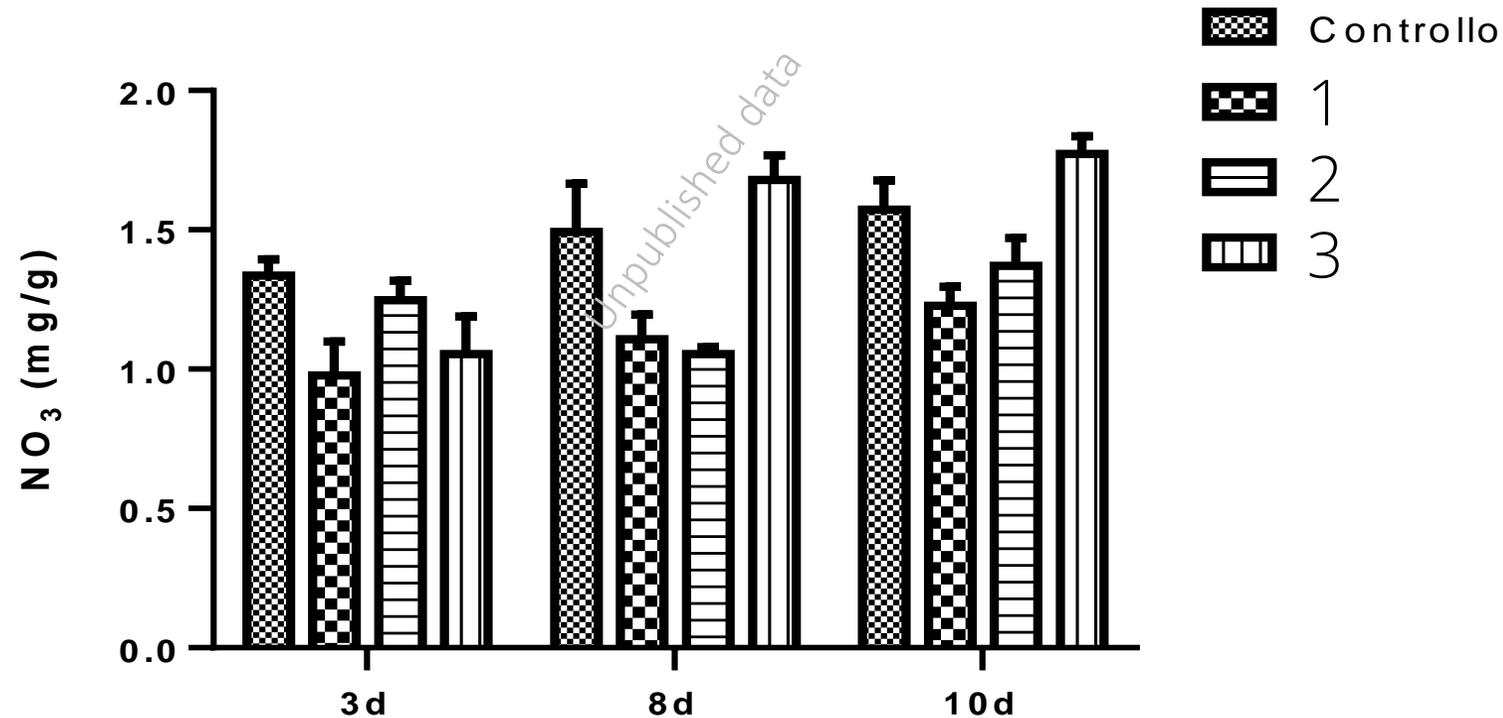
Franzoni, G., Cocetta, G., Prinsi, B., Ferrante, A., & Espen, L. (2022). Biostimulants on Crops: Their Impact under Abiotic Stress Conditions. *Horticulturae*, 8(3), 189.

Cocetta, G.; Landoni, M.; Pilu, R.; Repiso, C.; Nolasco, J.; Alajarin, M.; Ugena, L.; Levy, C.C.B.; Scatolino, G.; Villa, D.; Ferrante, A. Priming Treatments with Biostimulants to Cope the Short-Term Heat Stress Response: A Transcriptomic Profile Evaluation. *Plants* 2022, 11, 1130. <https://doi.org/10.3390/plants11091130>

# Analytical determinations

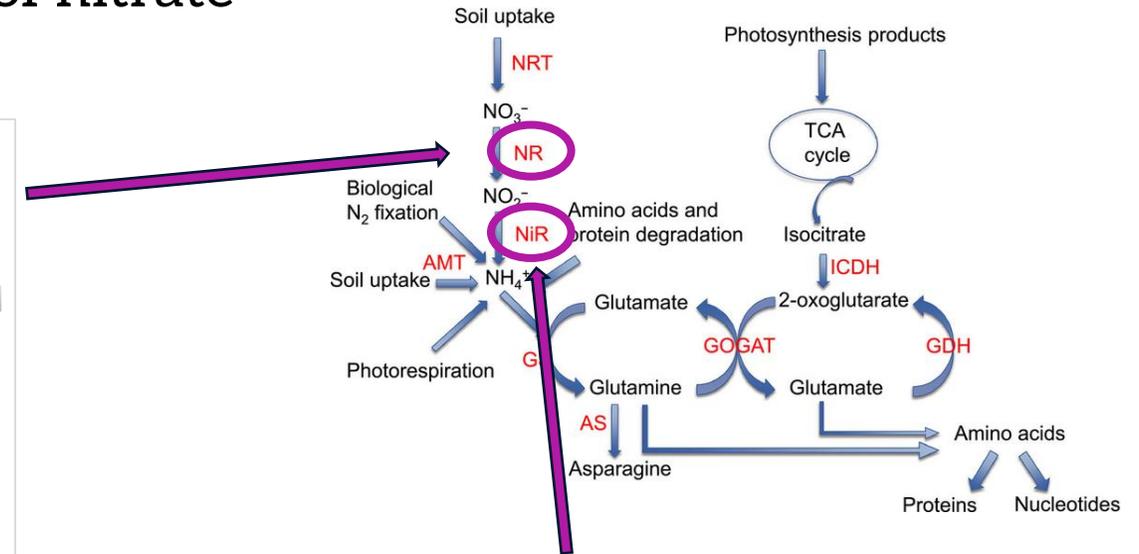
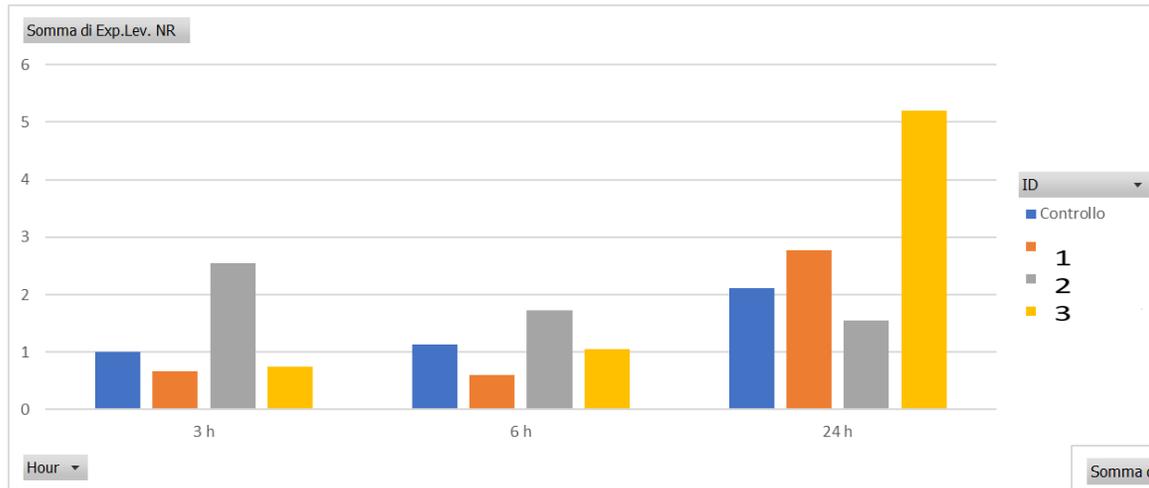


Mechanism of action: reduction of leaf nitrate level

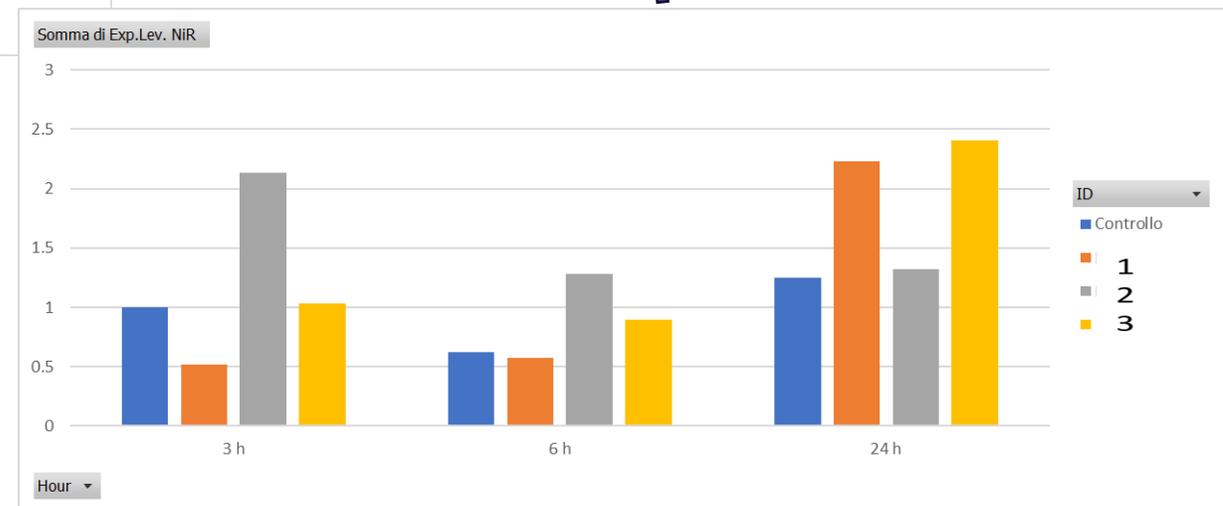


# Real Time PCR Gene expression

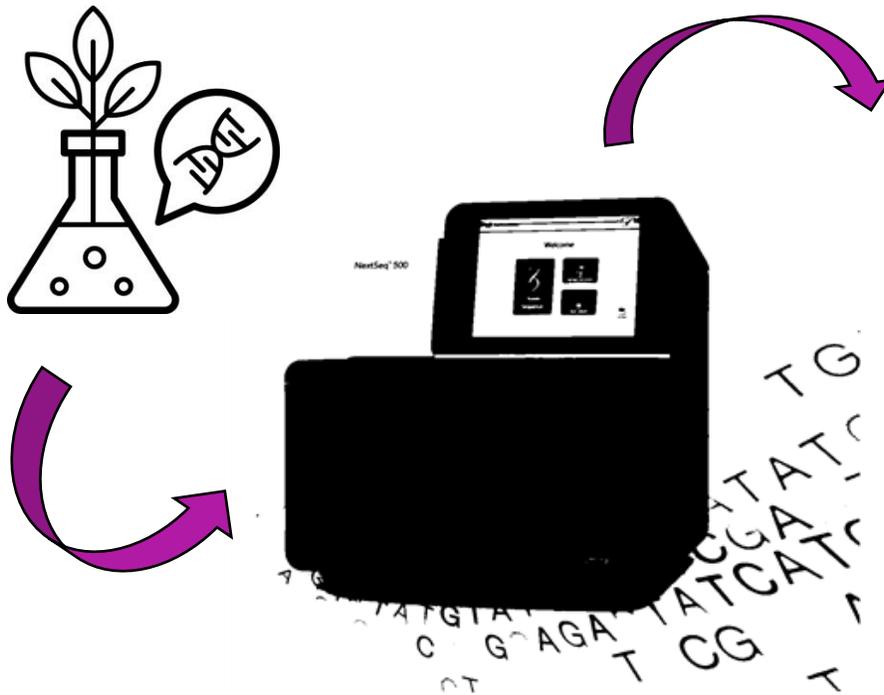
Mode of action: stimulation of key genes of nitrate metabolism.



Different products show different gene activation times.



# Transcriptome analysis (RNA seq)



We got 75,819 Unigenes.

Unigenes have been annotated by aligning with 7 functional databases, finally, 62,207 (NR:82.05%) , 70,218 (NT:92.61%) , 38,831 (Swissprot:51.22%) , 44,971 (KOG:59.31%) , 44,355 (KEGG:58.50%) , 44,311(GO:58.44%) , and 49,501 (InterPro:65.29%).

For functional annotation results, we detected 44,767 CDS. We also detected 2,526 Transcription Factor ( TF ) coding Unigenes.

# Transcriptome analysis (RNA seq)



Metabolic routes potentially affected by #3

## UP-REGULATED

Carbon metabolism and photosynthesis

Cell wall expansion

Nitrate reductase

Hormone-like responses (ethylene, gibberellins and auxins)

DNA damage repair

Aquaporins PIP1 and PIP2, proline and dehydration-sensitive proteins



## DOWN-REGULATED

NO<sub>3</sub>/Cl transporter suppression



## Part 2: New technologies to support the market positioning of biostimulant products

- Biostimulants and Nutrition Services: our approach
- Root growth
- Time-laps video
- Images analysis
- Roots and leaves scan
- Seed treatment
- Equipment for precision evaluations



# Biostimulants & nutrition services



## OUR APPROACH

We carry out experimental tests under different growth conditions, in a **protected environment** (growth chamber or greenhouse) as well as in the **open field**.



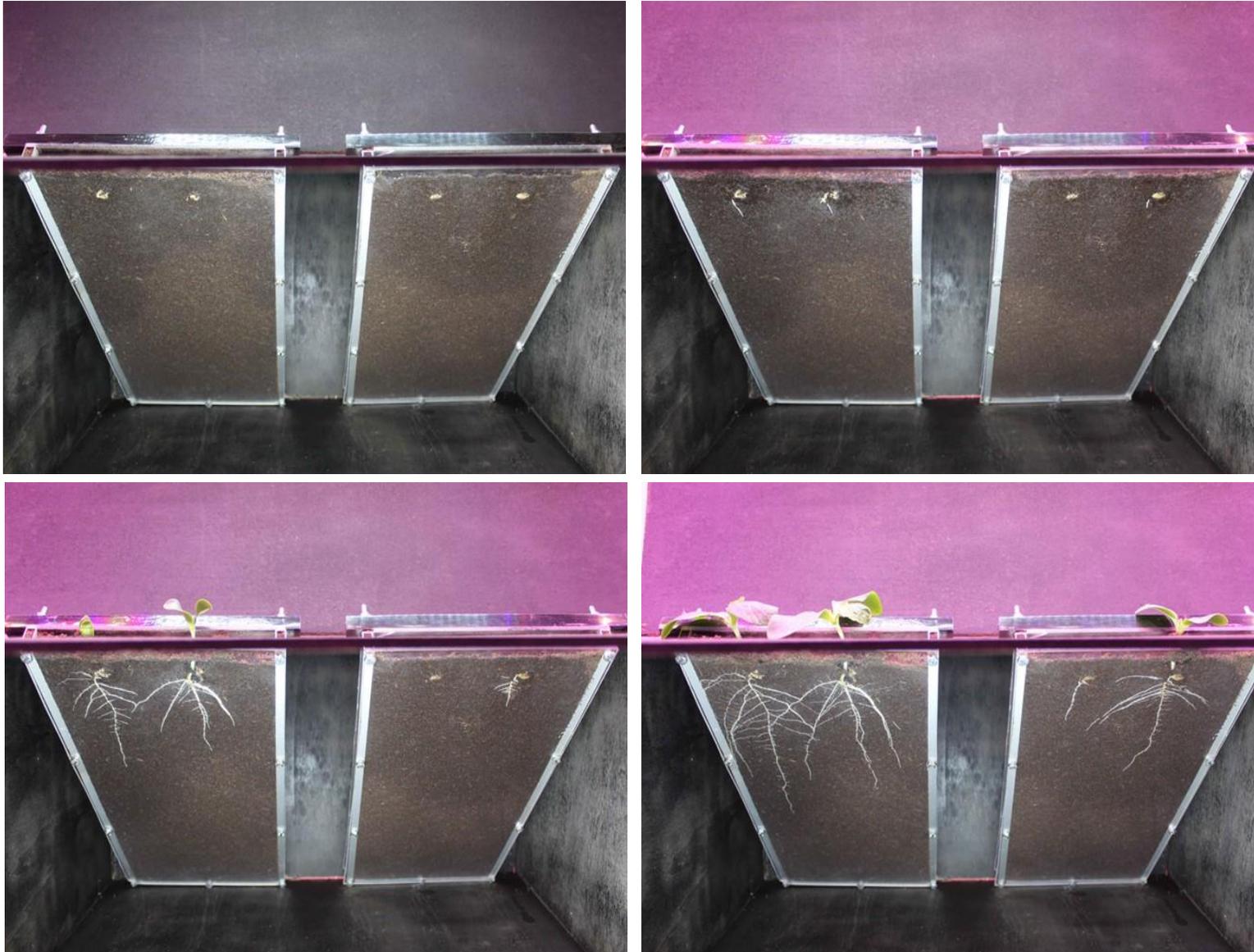
# Root growth



Thanks to a particular pot, called **rhizobox**, it is possible to evaluate the root system of several type of cultivated plants.



# Time-laps video



With professional instrumentation, we can process images and realise **time-lapse videos** to assess and monitor plant growth during the trial

# Images analysis

## 1. RGB

- Height
- Crop coverage
- Vigor

## 2. Multispectral (MSP)

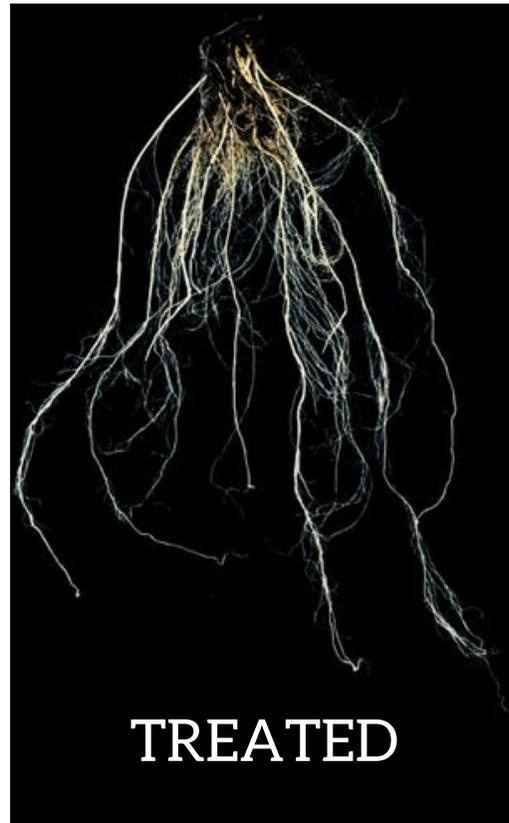
- NDVI
- NDRE
- Thermal camera

## Thermal images analysis

With thermal camera analysis it is possible to see stress situations when no symptoms are yet present on the plant

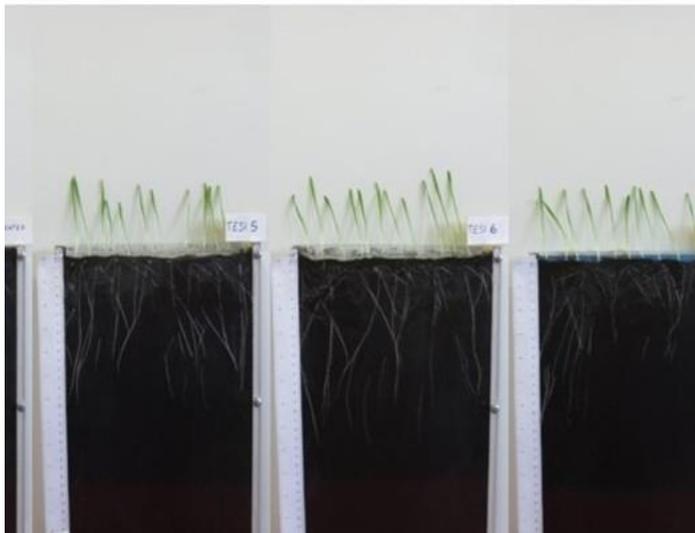


# Roots and leaves

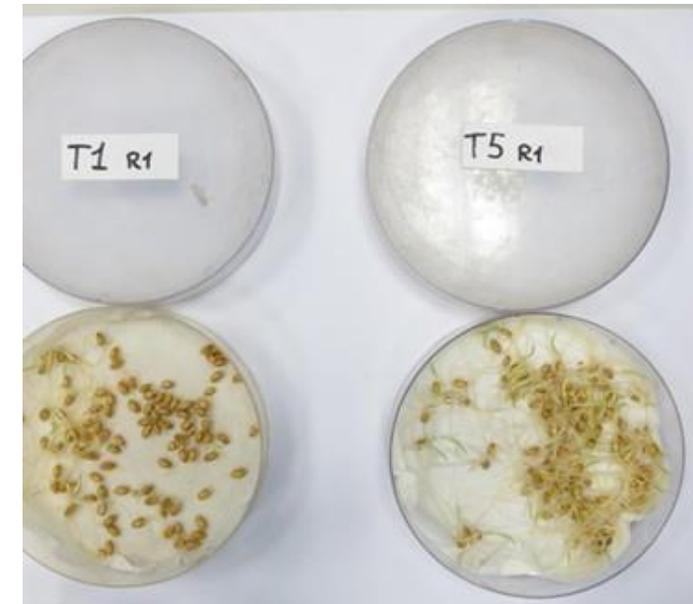


When roots have developed, we can scan the roots and the leaves of each plant and process the images in order to **calculate their surface**.

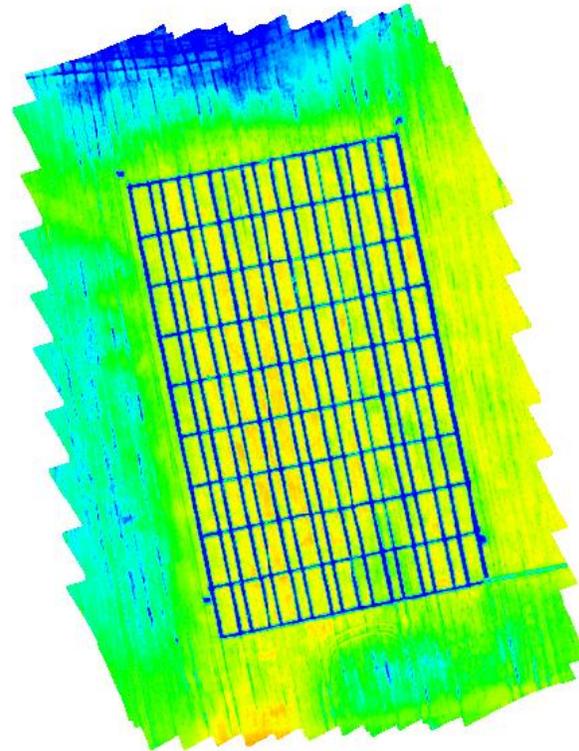
# Seed treatment



We can **evaluate the germination** of different seeds in Petri dishes, assuring all the growing conditions required, and performing seed treatment.



# Equipment for precision evaluations



Agricola 2000 have tools for obtaining multispectral maps to evaluate the vegetation status of the crops (non-destructive analysis)

Drone:

- DJI Phantom 4 RTK and Multispectral
- SPAD and NDVI



# Part 3:

## The importance of partnerships: the BIOSTIMOLA project and public-private collaboration

- Collaboration UNIMI - Agricola 2000
- BIOSTIMOLA project



# Collaboration



The collaboration between Agricola 2000 and the Department of Agriculture and Environment Science of the University of Milan, established in 2016, offers a broad knowledge of biostimulant products and the testing and evaluation of their efficacy, from the laboratory to the field.



# BIOSTIMOLA project



Regione  
Lombardia

Serie Ordinaria n. 39 - Mercoledì 28 settembre 2022

## D.G. Agricoltura, alimentazione e sistemi verdi

D.d.s. 23 settembre 2022 - n. 13466

Programma di Sviluppo Rurale 2014 - 2020 della Lombardia.  
operazione 1.2.01 «Progetti dimostrativi e azioni di informazione» d.d.s. 28 dicembre 2021, n. 18769. Approvazione degli esiti istruttori e ammissione a finanziamento delle domande

## BIOSTIMOLA: Biostimulants in a nutshell

A project to promote the use of biostimulants in agriculture

# BIOSTIMOLA project



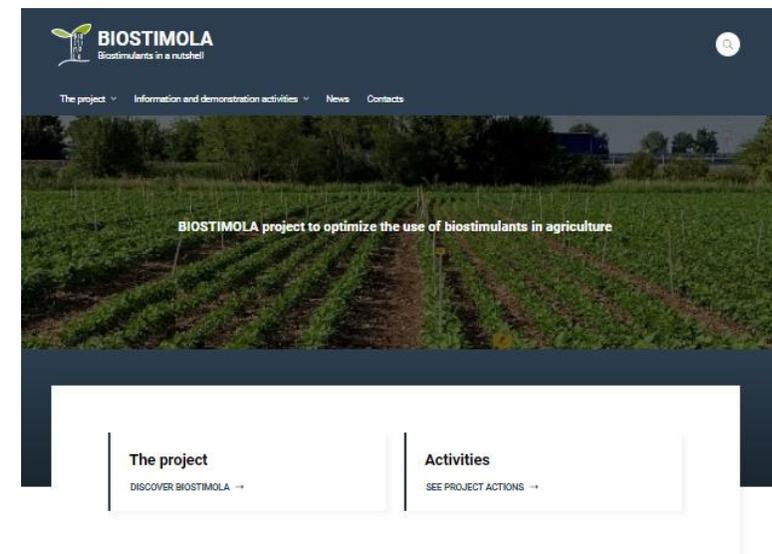
BIOSTIMOLA: Biostimulants in a nutshell

The information generated, collected and disseminated as part of the project will serve as support to those who will benefit from the use of biostimulants and provide them with technical and application knowledge regarding product selection, application methods, timing, dosages and objective assessment of crop responses, treated in different growing contexts.

## ACTION AND ACTIVITIES

- The demonstration and study days
- Information activities

<https://biostimola.unimi.it/en/>



# Conclusions



Services &  
Research  
for Agriculture



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We can identify the mode of action of biostimulant products.

**Multidisciplinary approaches** can be adopted to obtain a complete picture of the effects of the products and to maximize their efficacy (timing, dosage and application methods);

A continuous comparison with field agronomic evaluations and manufacturing process is essential to guide laboratory analyses and to correctly interpret the results obtained, in order to enhance and improve the design and use of new products.



# Thank You!

## Questions or Comments?

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