WHERE SCIENCE SERVES NATURE

Focus on Valagro’s investigation strategies for innovative biostimulant solutions

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Valagro
Where science serves nature

GLOBAL RESEARCH | SCENARIO

PRODUCING MORE

USING LESS RESOURCES

SATISFY THE INCREASED DEMAND FOR FOOD

FACE THE SCARCITY OF RESOURCES, INCREASING ITS EFFICIENCY

REDUCE THE IMPACTS OF AGRICULTURAL PRACTICES ON ENVIRONMENT

ENSURE SUSTAINABILITY IN THE LONG TERM

PLANT BIOSTIMULANTS

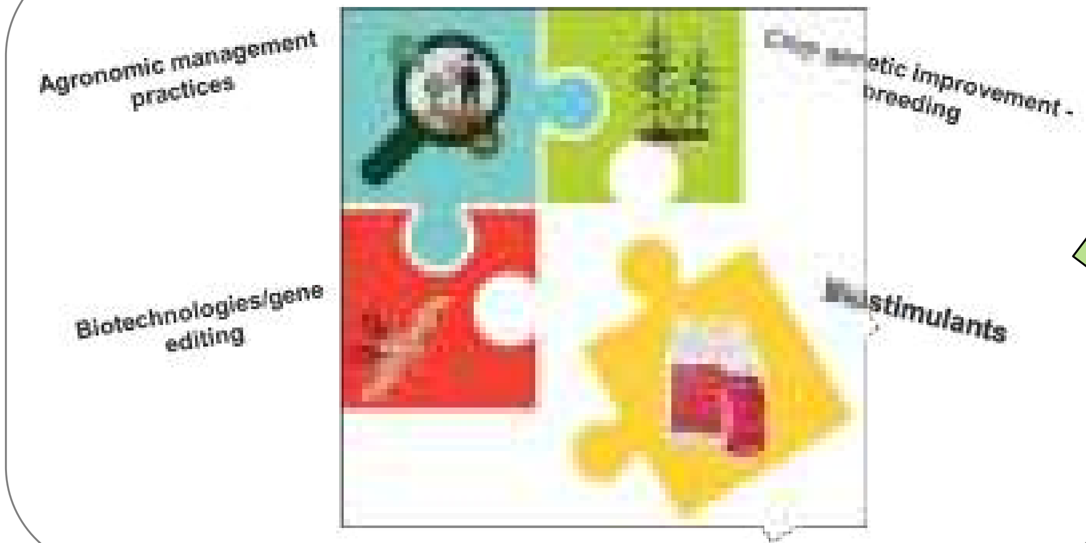
GLOBAL RESEARCH | PLANT BIOSTIMULANTS

Agronomic management practices

Crop genetic improvement - breeding

Biotechnologies/gene editing

GLOBAL RESEARCH | PLANT BIOSTIMULANTS



"Precision farming"/AgTec/DSS

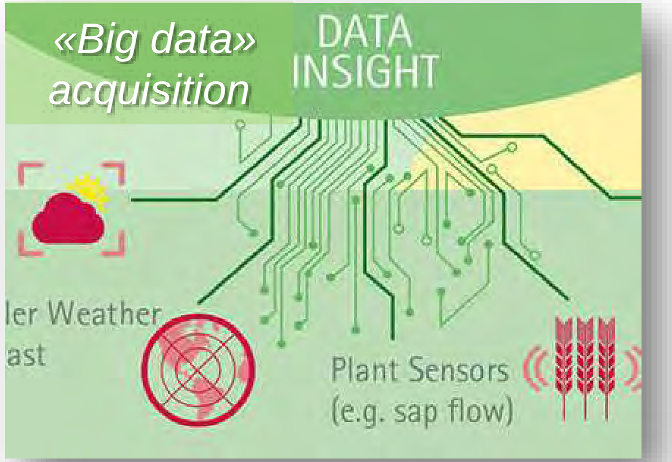
Management strategy that aims at carrying out agronomic interventions taking into account the **real cultivation need**, derived from the consideration that the **type of soil**, its **composition** and **microclimate** vary in different areas of a field in a non-linear and not easily observable way

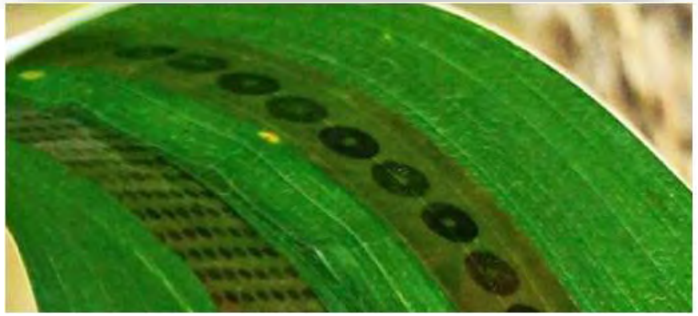
Precision agriculture involves the application of the right treatment in the right place at the right time

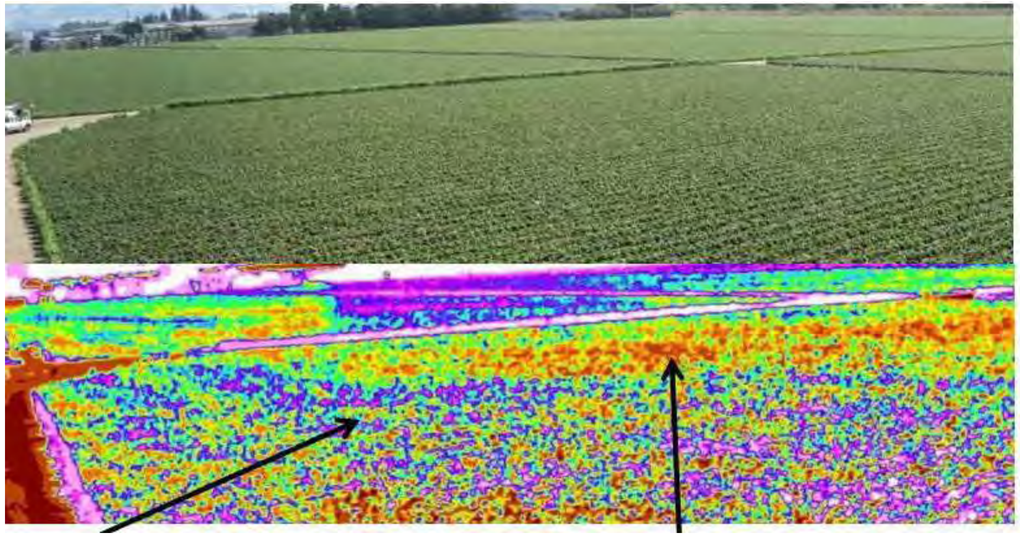
(Srinivasan, 1999; Robert, 2002; Stafford, 2006; Gebbers and Adamchuck, 2010)

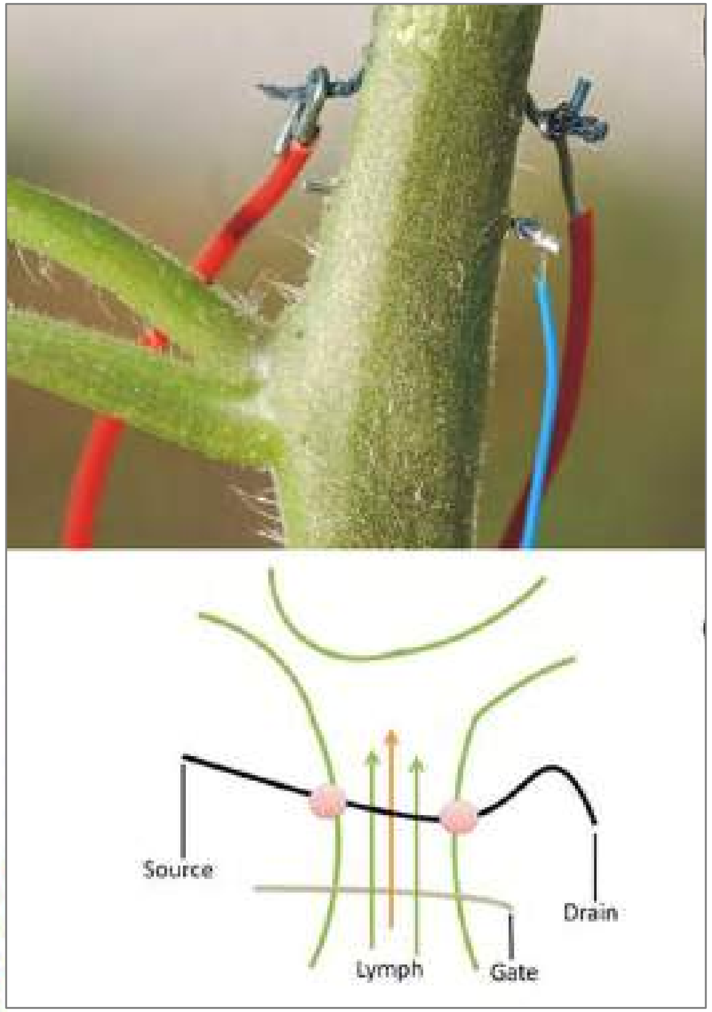
GLOBAL RESEARCH | PLANT BIOSTIMULANTS

Forecasting models for plant condition, early stress detection and stress monitoring









Coppedè et al., 2017

GLOBAL RESEARCH | PLANT BIOSTIMULANTS

Agronomic management practices

Crop genetic improvement - breeding

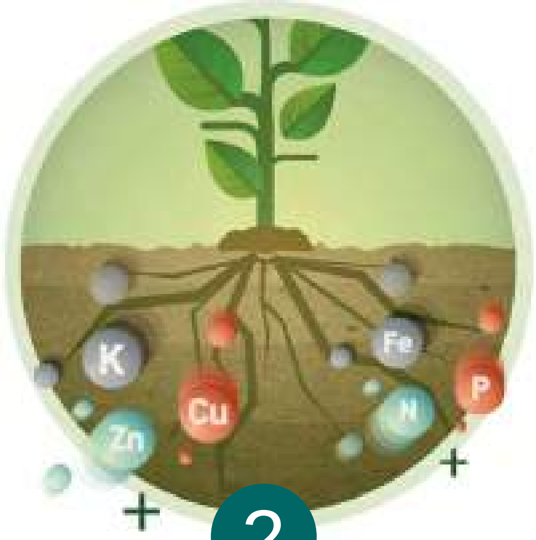
Biotechnologies/gene editing

Biostimulants

GLOBAL RESEARCH | PLANT BIOSTIMULANTS

1

Improve tolerance to
ABIOTIC STRESS



2

Improve
NUTRIENT USE EFFICIENCY

3

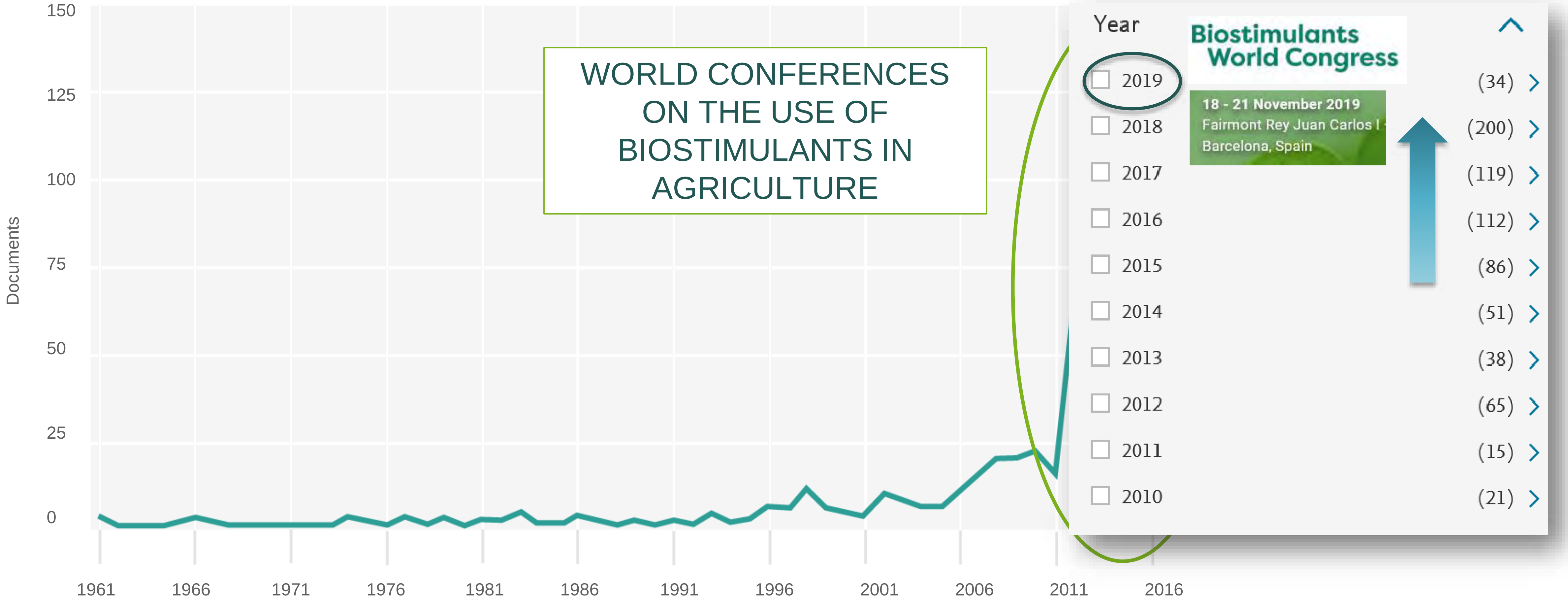
Improve
CROP QUALITY

«Plant Biostimulants contain substance(s) and/or microorganisms, whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality» (current EBIC definition)

*Biostimulants have no direct action against pests, and therefore **do not fall** within the regulatory framework of pesticides!*

GLOBAL RESEARCH | PLANT BIOSTIMULANTS

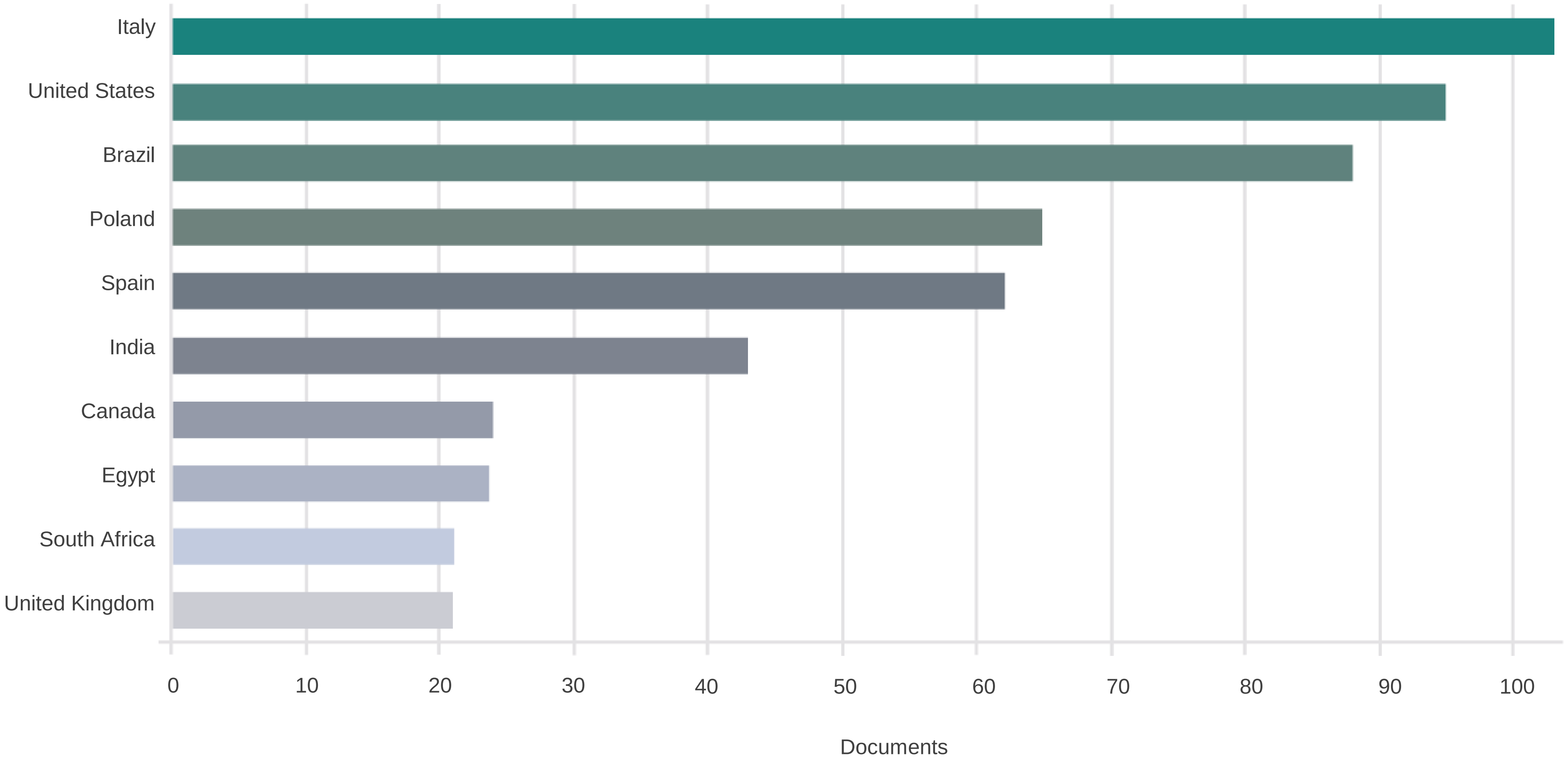
SCIENTIFIC PAPERS USING THE WORD "BIOSTIMULANT" (Title, abstract, keywords)



Scopus

GLOBAL RESEARCH | PLANT BIOSTIMULANTS

TOP 10 COUNTRIES IN PAPERS USING THE WORD “BIOSTIMULANT”



GLOBAL RESEARCH | PLANT BIOSTIMULANTS

Fruit
- Setting processes
- Fruit size and weight
- Quality

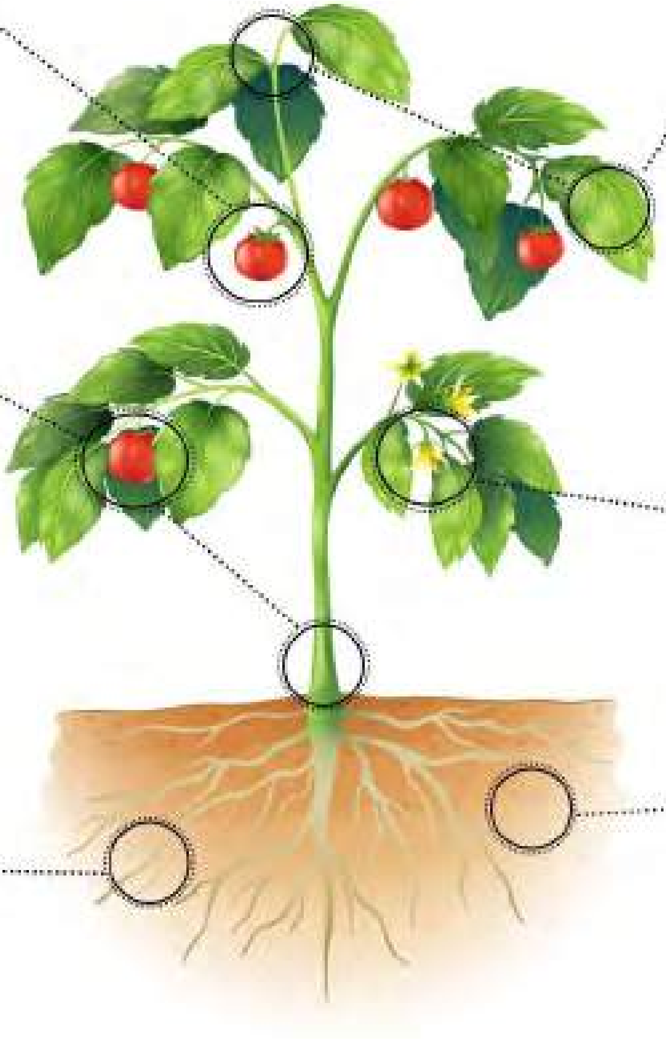
Crouch and van Staden, 1992; Chouliaras et al., 1997; Colapietra and Alexander, 2006; Basak, 2008; Chouliaras et al., 2009; Ross and Holden, 2010; Loyola and Muñoz, 2011; Parađiković et al., 2011; Khan et al., 2012; Parađiković et al., 2013; El-Hamied et al., 2015.

Seeds / Seedlings
- Germination
- "Starter effect"
- Overcoming transplant stress
- Priming effect
- Seed quality

Aldworth and van Staden, 1987; Featonby-Smith and van Staden, 1987; Crouch and van Staden, 1992; Russo et al., 1993; Moller and Smith, 1998; Demir et al., 2006; Sivasankari et al., 2006; Farooq et al., 2008; Neily et al., 2010; Kumar and Sahoo, 2011; Matysiak et al., 2011; Kalaivanan and Venkatesalu, 2012.

Roots
- Root development
- Young root development
- Rooting of cuttings

Sivasankari et al., 2006; MacDonald et al., 2010; De Lucia and Vecchietti, 2012; Ferrante et al., 2013; Krajnc et al., 2012; Petrozza et al., 2012; MacDonald et al., 2012; Alam et al., 2014.



Plant
- Plant growth/yield and physiological modulation
- Water/nutrient uptake
- Stress response

Beckett and van Staden, 1990; Beckett et al., 1994; Blunden et al., 1996; Adani, 1998; Mancuso et al., 2006; Zhang and Ervin, 2008; Ross and Holden, 2010; Sangeetha and Thevanathan, 2010; Zhang et al., 2010; Fan et al., 2011; Kumar and Sahoo, 2011; Matysiak et al., 2011; Parađiković et al., 2011; De Lucia and Vecchietti, 2012; Petrozza et al., 2012; Parađiković et al., 2013; Alam et al., 2014; Petrozza et al., 2014; Saa et al., 2015.

Flowers
- Flowering and sprouting induction.

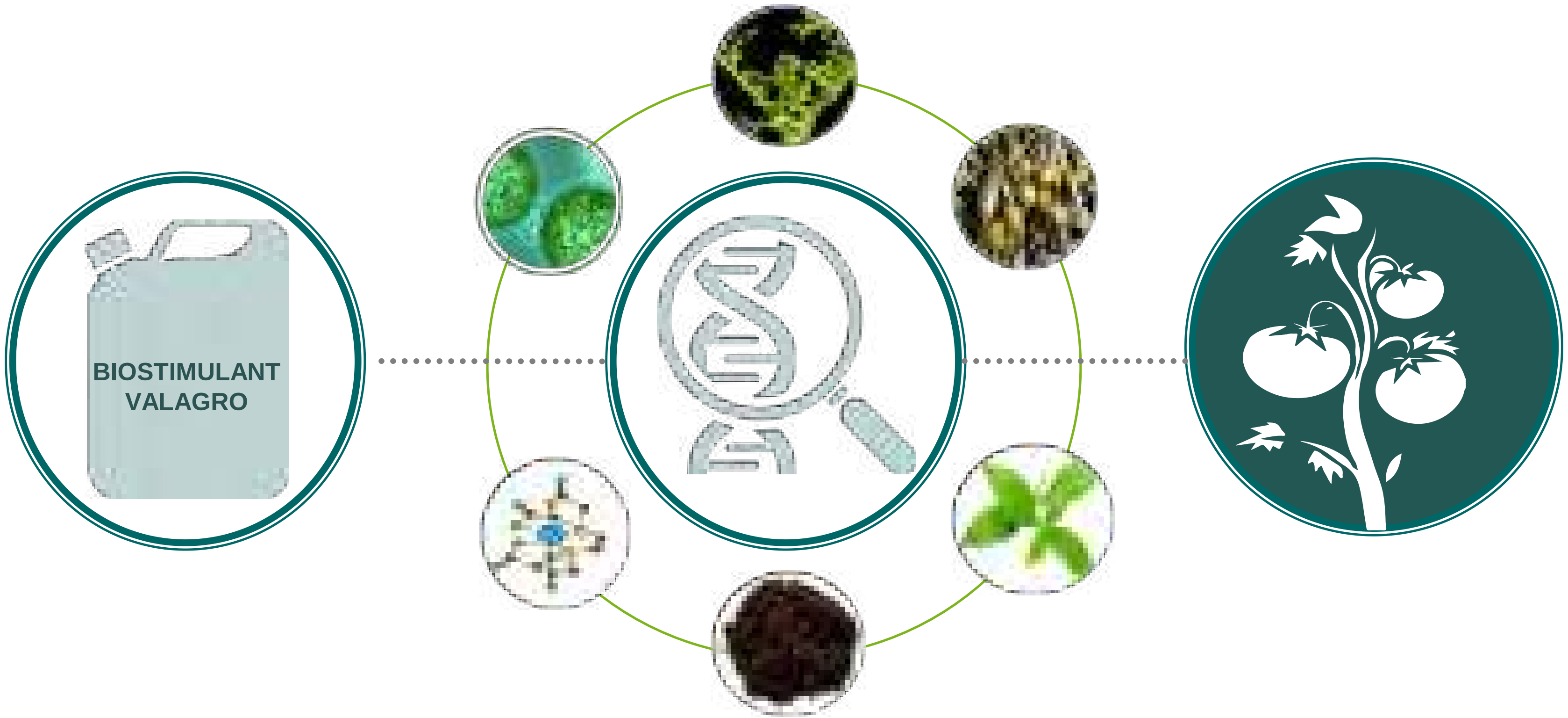
Basak, 2008; Petri et al., 2008; Hawerroth et al., 2010; Pereira et al., 2011.

Soil
- Physico-chemical properties
- Development of beneficial soil microorganisms
- Water/nutrient retention
- Overcoming salinity stress

Booth, 1969; Guiry and Blunden, 1991; Temple and Bomke, 1988; Chen et al., 2002; Gulser et al., 2010; Ross and Holden, 2010; García-Martínez et al., 2010; Tejada et al., 2011; Alam et al., 2014.

GLOBAL RESEARCH TECHNOLOGY PLATFORM

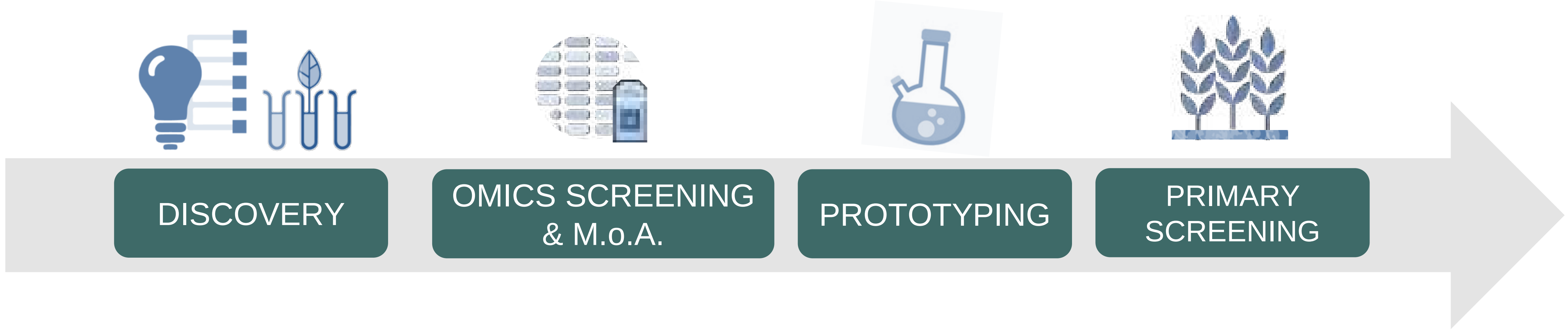
GLOBAL RESEARCH | TECHNOLOGY PLATFORM



The knowledge on the benefits of Plant BioStimulants is constantly improving (consistent increase of research papers). Less is known about their “**Mode Of Action**”.

NATURAL BUT COMPLEX MATRICES: WHAT MAKES THEM SO «SPECIAL»?

GLOBAL RESEARCH | TECHNOLOGY PLATFORM



GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY → OMICS SCREENING → PROTOTYPING → PRIMARY SCREENING

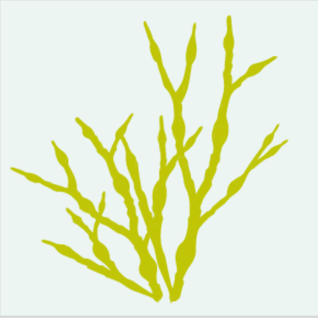
Business Concept
(Innoteam)

↓

DISCOVERY →

ANALYSIS OF AN UNMET NEED

EXPLORATION/ LITERATURE SEARCH



SCOUTING CANDIDATES

BIOASSAYS

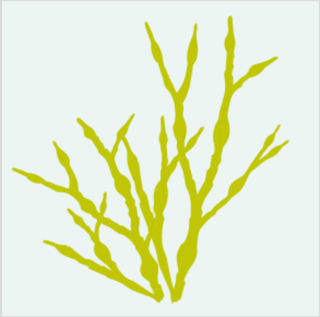
STUDY OF EXTRACTION OR FERMENTATION PROCESSES

↓

WORKING HYPOTHESIS

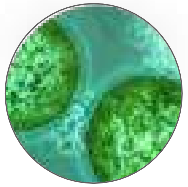
GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY → OMICS SCREENING → PROTOTYPING → PRIMARY SCREENING

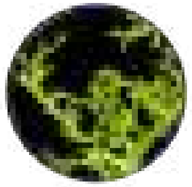


SCOUTING CANDIDATES

MICROBIALS (AND DERIVATIVES)



MICROALGAE



SEAWEEDS

PHYTOCHEMICALS & PLANT EXTRACTS

ACTIVE INGREDIENTS

FORMULA PLATFORM

- TYPOLOGICAL
- TECHNICAL SHEET
- FORMULA SOLUTION

OUTCOME

Selected raw materials, microorganisms, extracts, molecules to satisfy the need

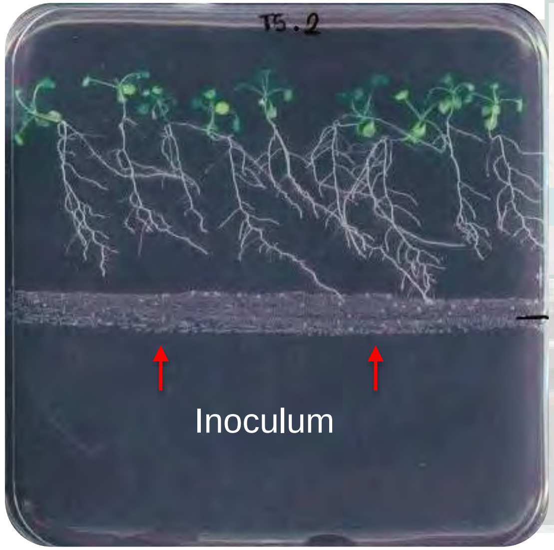
GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY → OMICS SCREENING → PROTOTYPING → PRIMARY SCREENING

Speed up preliminary screening process: no soil and competition with fungi/bacteria

BIOASSAYS

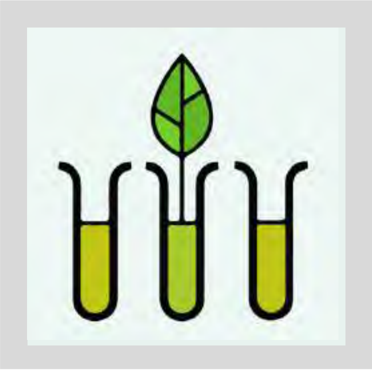
- Selection of the best **model plant** (Arabidopsis, tomato, Brachipodium, *etc.*)
- Plants germination and growth under sterile conditions, on liquid or solid nutrient medium
- **Light and temperature parameters** are modulated/monitored
- Plant Biostimulants are added to evaluate **dose-effect response curve**
- Also studies of **microbials effect** on plants (PGPR, nutrient solubilization)



GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY → OMICS SCREENING → PROTOTYPING → PRIMARY SCREENING

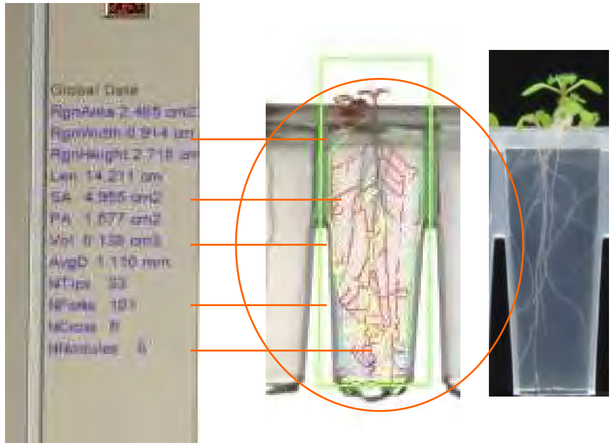
From Qualitative to Quantitative (microphenotyping)…



BIOASSAYS

UTC | Stress | Biostimulant | Stress + Biostimulant

WinRHIZO™
Analysis of Washed Roots and Arabidopsis Seedlings



Software-assisted root image analysis

OUTCOME → Pre-selection and characterization of physiological activity

GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY → OMICS SCREENING → PROTOTYPING → PRIMARY SCREENING

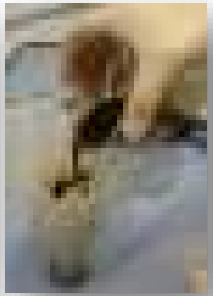
STUDY OF EXTRACTION OR FERMENTATION PROCESSES

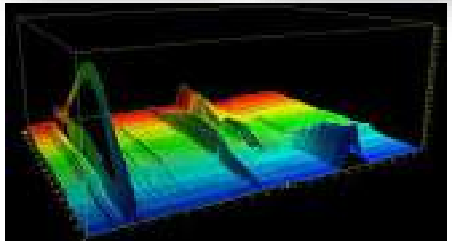
- Testing of **different extraction procedures**, selecting the best ones in terms of process and yield

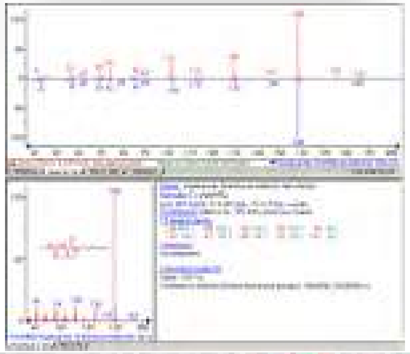
- Extraction procedures are calibrated in order to **selectively isolate categories of chemicals** specific for the intended use, utilizing appropriate solvent mixtures, pH, temperature and eventually enzymes to drive the process

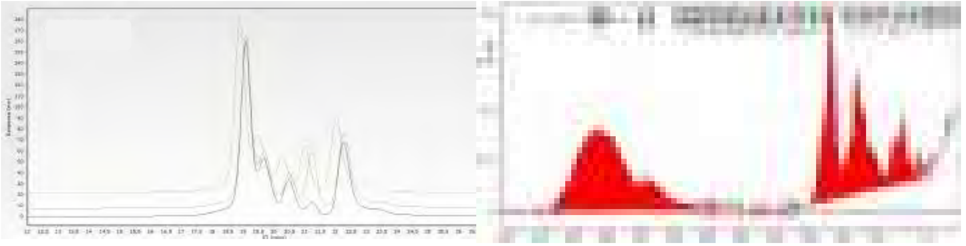
- Evaluation of the outcome; **quali-quantitative analysis** of the active ingredients

- **Scaling-up** from mg to grams







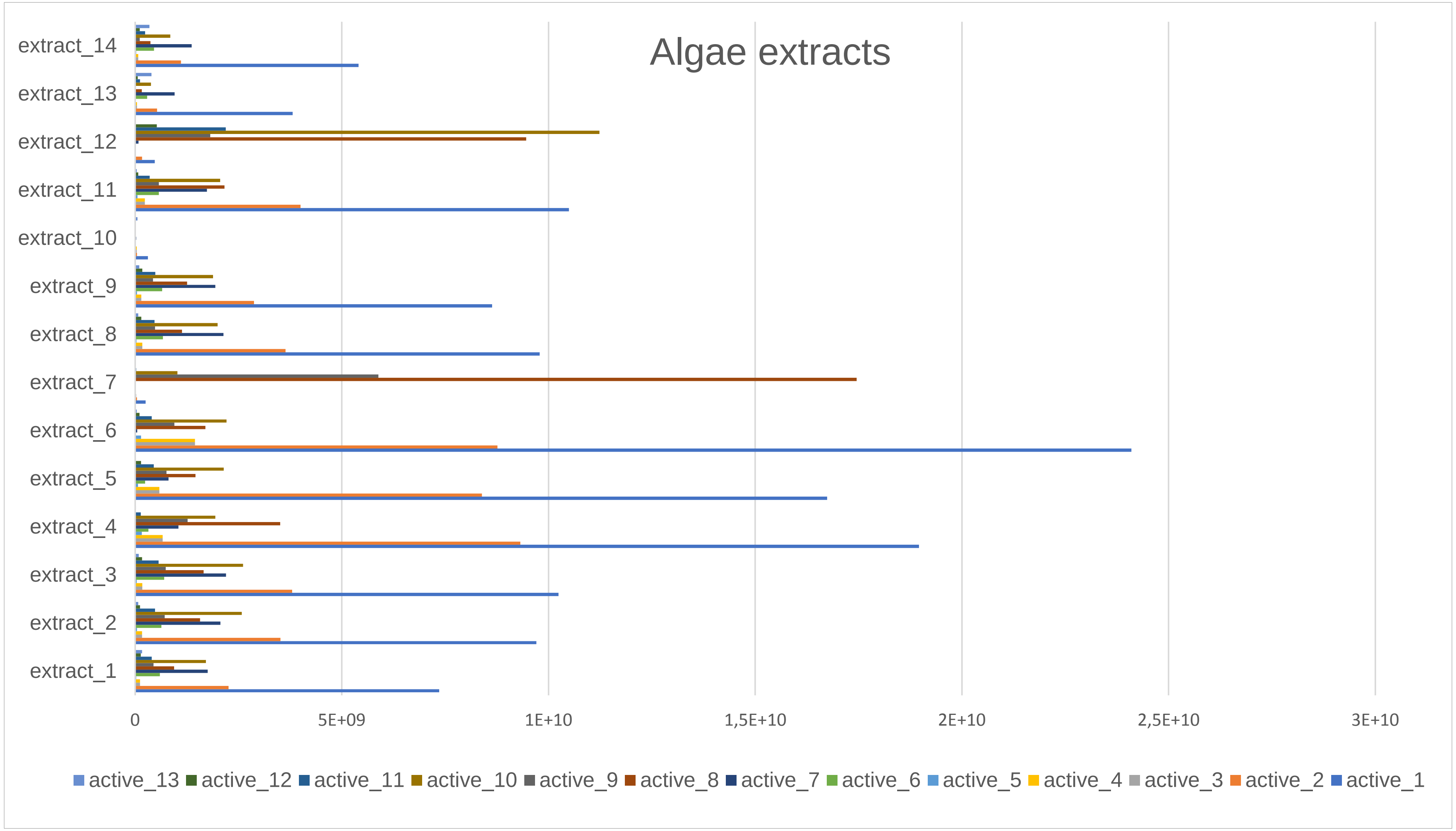


OUTCOME: best extract to be formulated

GLOBAL RESEARCH | TECHNOLOGY PLATFORM

Example. **Process optimization** to maximize the yield of low-weight molecular **actives**

STUDY OF EXTRACTION OR FERMENTATION PROCESSES



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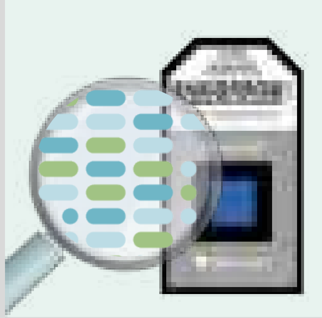
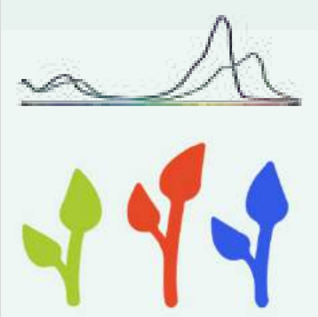
DISCOVERY → **OMICS SCREENING** → PROTOTYPING → PRIMARY SCREENING

OMICS SCREENING & M.o.A.

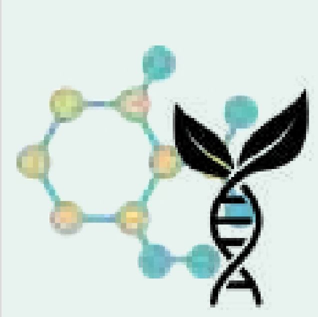


TRANSCRIPTOMICS



PHENOMICS

METABOLOMICS



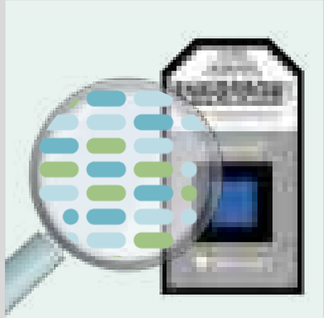
OTHER OMICS/METHODS

PRELIMINARY SELECTION & EFFICACY CHARACTERIZATION (*mode of action*)

GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY → **OMICS SCREENING** → PROTOTYPING → PRIMARY SCREENING

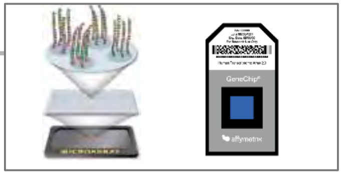
Transcriptomics as powerful tool to decipher the molecular/physiological triggers for specific responses



TRANSCRIPTOMICS

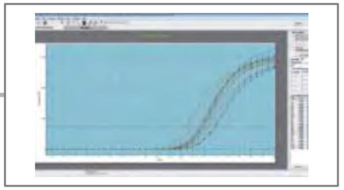
Microarray, qPCR and Next Gen. Sequencing

EXTRACTION



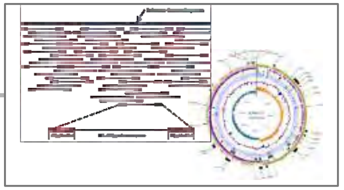
Gene chip

Microarray: high-throughput technology to measure parallel expression of thousands of genes



Real-time qPCR analysis

«Real-time» qPCR to focus on specific physiological processes/pathways or validate microarray data



NGS

Next Generation Sequencing: genomics «on field», dedicated to non-model plants & microorganisms

OUTCOME: Molecular dissection of the effect of biostimulants and explanation of the mode of action

GLOBAL RESEARCH | TECHNOLOGY PLATFORM

Gene-chip microarray

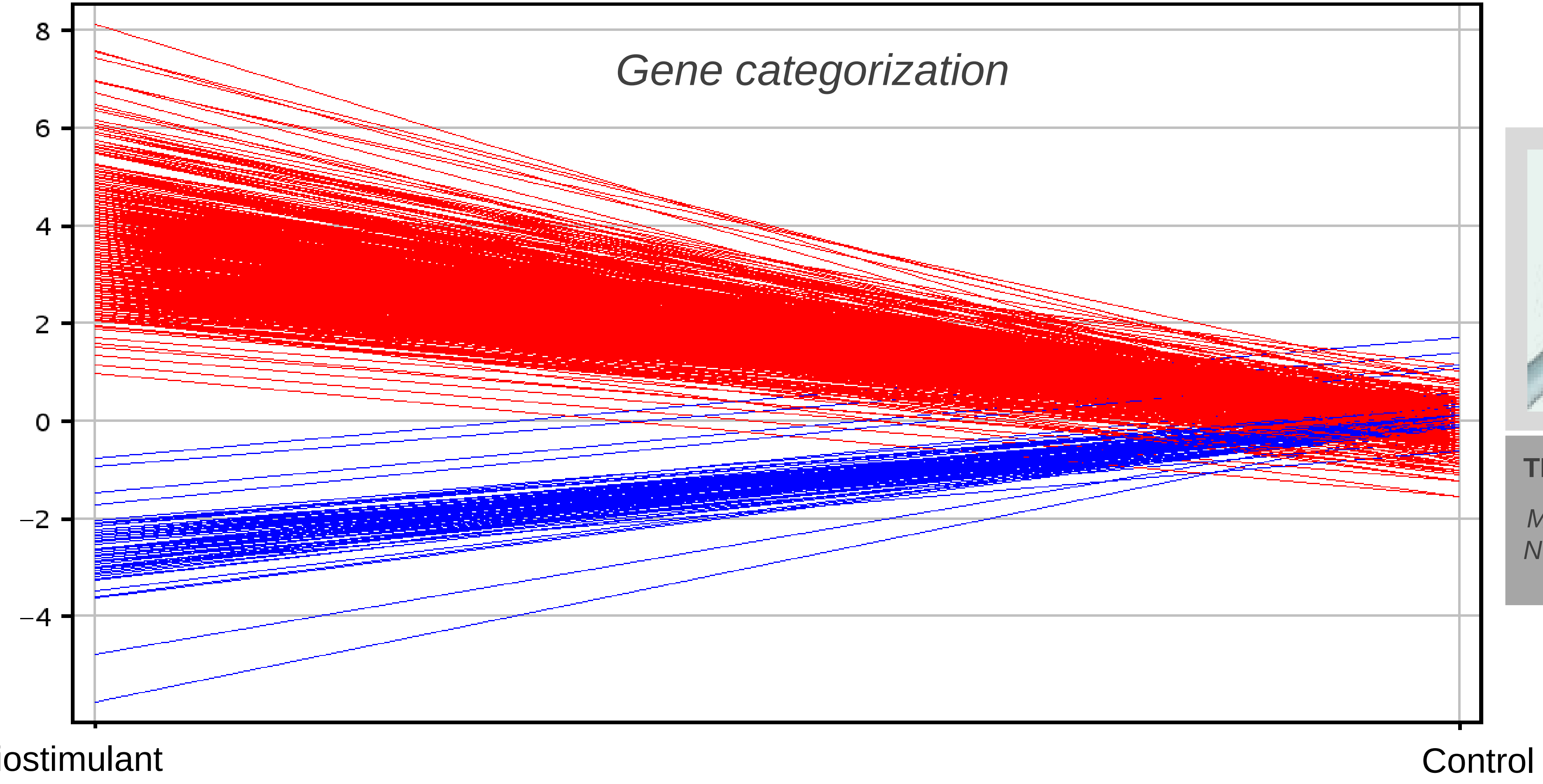
DISCOVERY | OMICS SCREENING | PROTOTYPING | PRIMARY SCREENING

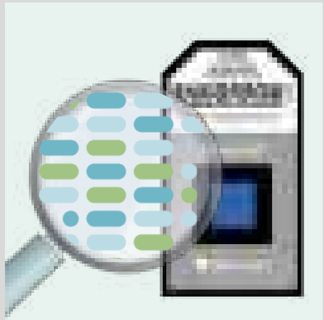
GLOBAL RESEARCH | TECHNOLOGY PLATFORM

Gene-chip microarray

Up-regulated genes

Down-regulated genes



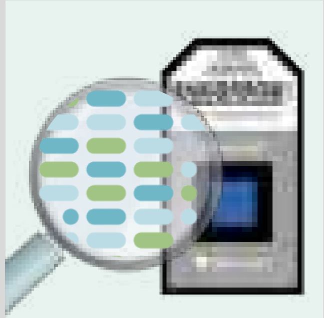


TRANSCRIPTOMICS

Microarray, qPCR and Next Gen. Sequencing

Gene-chip microarray

Example. Overview/fingerprint of the Arabidopsis transcriptome in response to **Megafol®** compared to untreated test



TRANSCRIPTOMICS

Microarray, qPCR and Next Gen. Sequencing

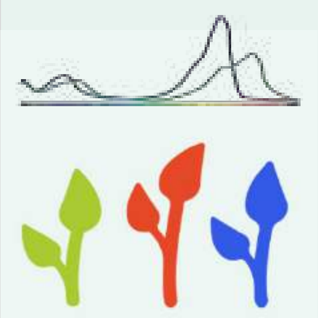
Locus Identifier	AnnotationAnnotationAnnotationAnnotationAnnotation	FUNCTION	MEGAFOL F
AT4G10270	wound-responsive family proteinwound-responsive family protein	STRESS wound	62
AT3G10040	transcription factortranscription factortranscription factor	STRESS anoxia	46
AT3G02550	LOB domain protein 41 / lateral organ boundaries domain protein 41	STRESS biotic eFP	33
AT4G33070	pyruvate decarboxylase, putativepyruvate decarboxylase, putative	STRESS anoxia	25
AT2G37870	protease inhibitor/seed storage/lipid transfer protein (LTP) family prot	STRESS salt eFP	18
AT5G09520	hydroxyproline-rich glycoprotein family protein	HORMONE ABA eFP	17
AT4G33560	similar to wound-responsive protein-related [Arabidopsis thaliana] (TA	STRESS wound	16
AT1G77120	ADH1 (ALCOHOL DEHYDROGENASE 1); alcohol dehydrogenase	STRESS anoxia	14
AT2G47780	rubber elongation factor (REF) protein-related	STRESS salt eFP	10
AT5G04120	phosphoglycerate/bisphosphoglycerate mutase family protein	METABOLISM	10
AT5G62520	SRO5 (SIMILAR TO RCD ONE 5); NAD+ ADP-ribosyltransferase	STRESS cold wound eFP	8
AT5G13900	protease inhibitor/seed storage/lipid transfer protein (LTP) family prot	HORMONE ABA eFP	8
AT1G76650	calcium-binding EF hand family protein	STRESS cold eFP	8
AT1G52690	late embryogenesis abundant protein, putative / LEA protein, putative	STRESS osmotic eFP	7
AT4G16780	ATHB-2 (Homeobox-leucine zipper protein HAT4); DNA binding / trar	STRESS cold eFP	7
AT4G36610	hydrolase, alpha/beta fold family protein	HORMONE ABA eFP	7
AT1G02930	[AT1G02930, ATGSTF6 (EARLY RESPONSIVE TO DEHYDRATION	STRESS drought	6
AT5G07010	sulfotransferase family proteinsulfotransferase family protein	STRESS wound eFP	5
AT5G59320	LTP3 (LIPID TRANSFER PROTEIN 3); lipid binding	STRESS osmotic salt eF	5
AT2G43620	chitinase, putativechitinase, putativechitinase, putative	STRESS osmotic eFP	5
AT1G72360	ethylene-responsive element-binding protein, putative	HORMONE ETHYLENE	5
AT3G13310	DNAJ heat shock N-terminal domain-containing protein	STRESS heat	5
AT5G45340	CYP707A3 (cytochrome P450, family 707, subfamily A, polypeptide 3	STRESS cold wound eFP	5
AT3G23170	similar to ATBET12 [Arabidopsis thaliana] (TAIR:AT4G14450.1)	STRESS cold eFP	5
AT1G19250	FMO1 (FLAVIN-DEPENDENT MONOOXYGENASE 1); monooxygen	STRESS biotic	5
AT2G34390	[AT2G34390, NIP2;1/NLM4 (NOD26-LIKE INTRINSIC PROTEIN 2;1)	STRESS anoxia	5
AT5G40590	DC1 domain-containing proteinDC1 domain-containing protein	HORMONE ETHYLENE e	4
AT5G22460	esterase/lipase/thioesterase family protein	STRESS osmotic eFP	4
AT3G02480	ABA-responsive protein-relatedABA-responsive protein-related	STRESS osmotic eFP	4
AT2G43570	chitinase, putativechitinase, putativechitinase, putative	STRESS osmotic eFP	4
AT2G47770	benzodiazepine receptor-relatedbenzodiazepine receptor-related	STRESS osmotic eFP	4
AT5G66400	RAB18 (RESPONSIVE TO ABA 18)	STRESS osmotic	4
AT4G37770	ACS8 (1-Amino-cyclopropane-1-carboxylate synthase 8)	HORMONE ETHYLENE	4
AT5G13580	ABC transporter family proteinABC transporter family protein	TRANSPORT	4
AT5G54490	PBP1 (PINOID-BINDING PROTEIN 1); calcium ion binding	HORMONE AUXIN	4
AT3G21720	isocitrate lyase, putativeisocitrate lyase, putative	METABOLISM	4
AT5G50260	cysteine proteinase, putativecysteine proteinase, putative	HORMONE ABA eFP	4
AT5G10230	ANN7 (ANN7, ANNEXIN ARABIDOPSIS 7); calcium ion binding / calc	HORMONE ABA eFP	4
AT4G33550	lipid bindinglipid bindinglipid bindinglipid bindinglipid binding	HORMONE ABA eFP	4
AT2G22510	hydroxyproline-rich glycoprotein family protein	HORMONE ABA eFP	4

127 up regulated genes fold >3

GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY | OMICS SCREENING | PROTOTYPING | PRIMARY SCREENI

High-throughput, multi-spectrum i… etric and physiological parameters



PHENOMICS
High throughput image analysis

In-house HTS phe

UV (fluorescence): to analyze the photosynthetic efficiency

Visible - RGB: morphology, architecture, digital biomass, green and yellow index

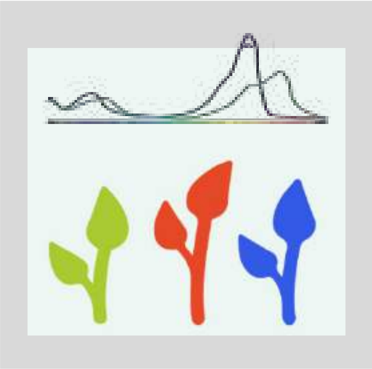
NIR (Near Infra-Red): plant water content

TCOME: Phenotype characterization of …ritional, hydrological, physiological state of plants

GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY → **OMICS SCREENING** → PROTOTYPING → PRIMARY SCREENING

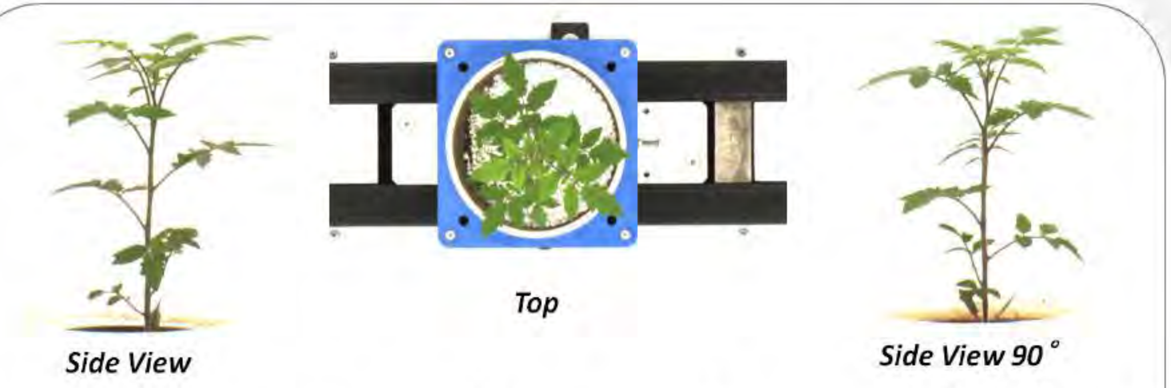
High-throughput, multi-spectrum image analysis to detect morphometric and physiological parameters



PHENOMICS
High throughput image analysis

https://www.youtube.com/watch?v=xj3-r9sJyZM

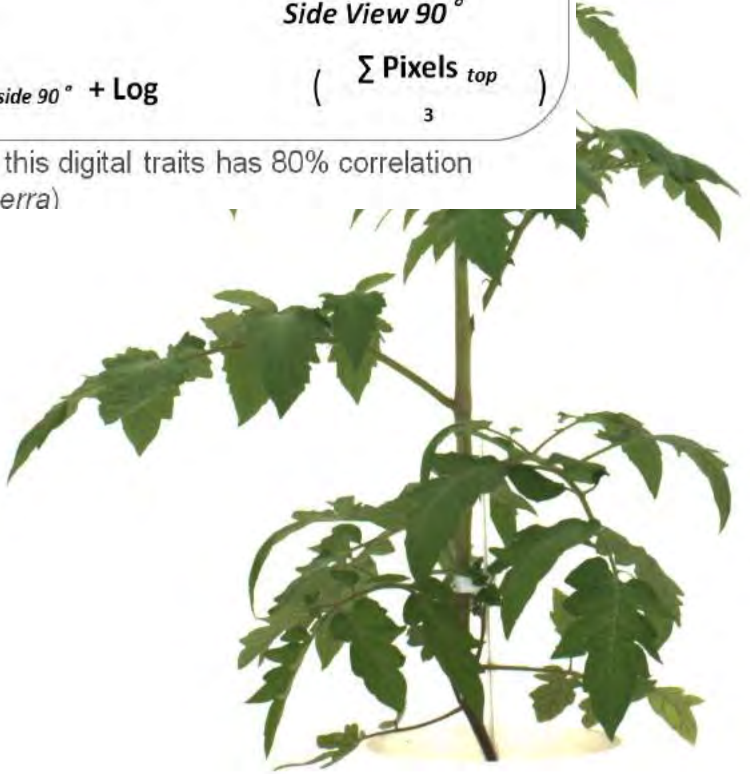
Example. RGB (Red-Green-Blue) → Digital biomass



$$\text{Digital\_biomass} = \sum \text{Pixels}_{\text{side view}} + \sum \text{Pixels}_{\text{side } 90^\circ} + \text{Log}\left(\frac{\sum \text{Pixels}_{\text{top}}}{3}\right)$$

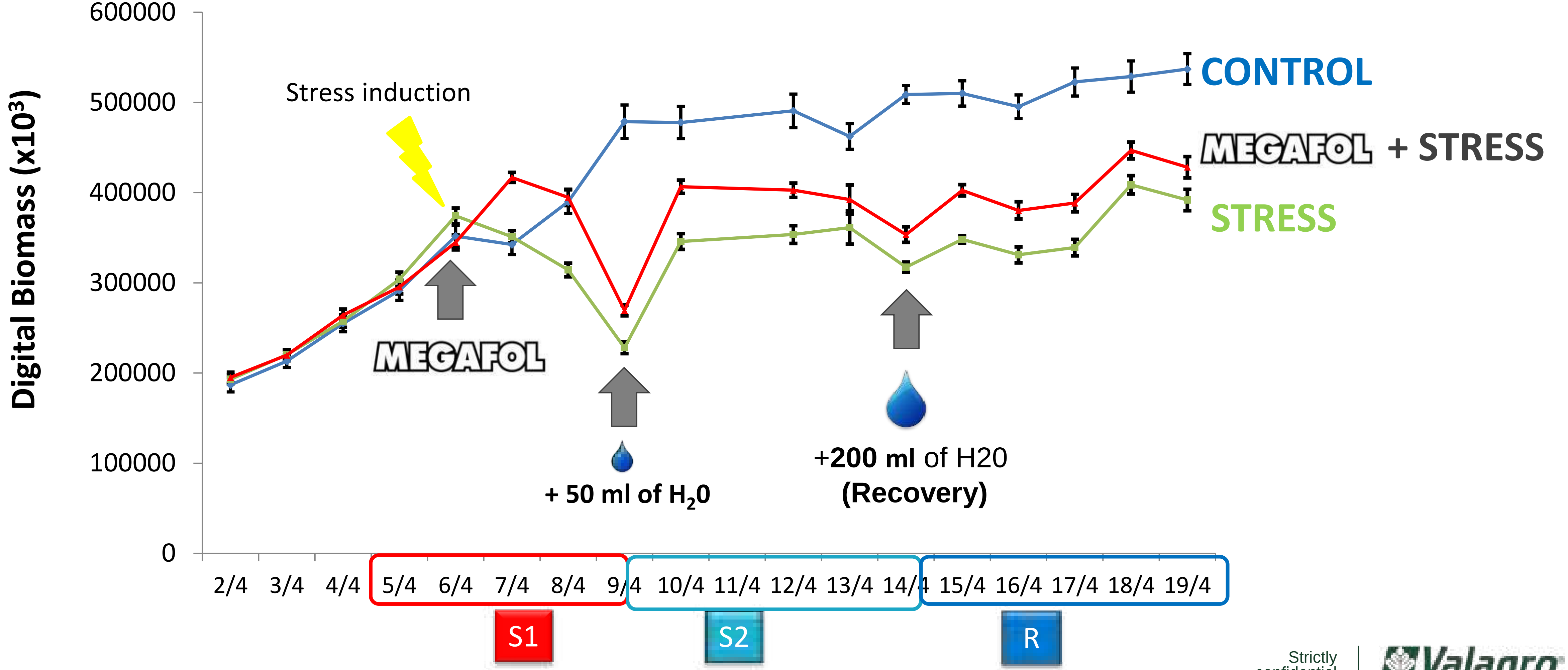
Destructive verification experiments show that this digital traits has 80% correlation with green mass (*KeyGene, R.v. Berloo, J. Guerra*)

UNTREATED



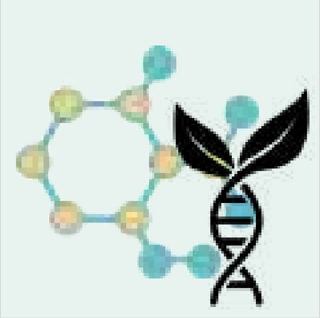
MEGAFOL

Example. RGB (Red-Green-Blue) → Digital biomass

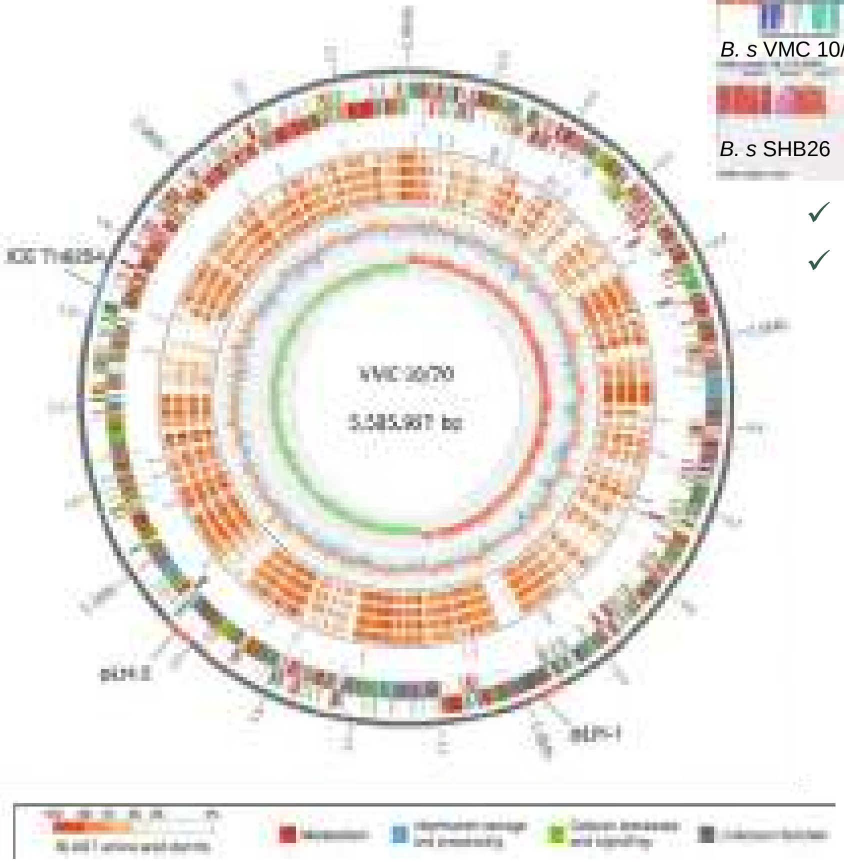


GLOBAL RESEARCH | TECHNOLOGY PLATFORM

DISCOVERY › OMICS SCREENING › PROTOTYPING › PRIMARY SCREENING



OTHER OMICS/METHODS



- ✓ Unicity of the strain for patent
- ✓ Discovery additional know/unkown PGP traits

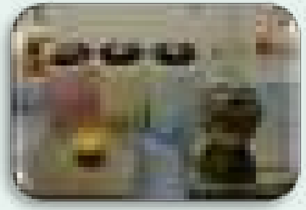
- Siderophore biosynthesis protein and iron transporter (gene cluster)
- Phosphates solubilization (*gdc* gene)
- Phosphonate metabolism and degradation (gene cluster)
- Activate nutrient up-take (under annotation)

Siderophore production | Zn solubilisation | P solubilisation

Genome Strain Characterization| VMC 10/70

GLOBAL RESEARCH | TECHNOLOGY PLATFORM

PROTOTYPING



FORMULATION DEVELOPMENT

STABILITY AND SHELF-LIFE

EASE OF USE TESTS

SCALING-UP

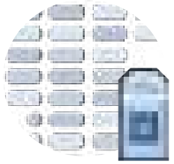
REALIZATION OF THE BEST PROTOTYPE

GLOBAL RESEARCH | PRIMARY SCREENING

DISCOVERY → OMICS SCREENING → PROTOTYPING → PRIMARY SCREENING

PLANT GROWTH CHAMBER

+ M.o.A. analysis/validation on final formulation, through omics



PRIMARY SCREENING

CONTROLLED ENVIRONMENT

PLOT TESTS OUTDOOR

BIOLOGICAL INCUBATOR

BEST APPLICATION METHODS, TIMING, RATES

GREENHOUSES

BIG POTS

Valagro
Where science serves nature

LEADING DEVELOPMENT CAPABILITY

GEAPOWER | TODAY FOR TOMORROW

GEAPOWER REDUCES THE COST OF TAKING A SOLUTION TO MARKET WHILE ENSURING CONSISTENT EFFICACY

1 DEEP KNOWLEDGE OF ACTIVE INGREDIENTS AND RAW MATERIALS

- This enables Valagro to identify, characterize and preserve specific active ingredients that can achieve targeted physiological responses in plants

2 PROPRIETARY EXTRACTION PROCESSES

- Customized extraction processes help maintain the correct ratio of each ingredient in complex natural mixtures

3 ADVANCED SCREENING AND INVESTIGATION TECHNOLOGIES

- Genomics, phenomics and other “omic” sciences allow Valagro to decipher the genetic and molecular triggers for specific physiological responses in plant systems.
- Screening of hundreds of samples per experiment.

4 PROVEN ABILITY TO PROVIDE COMMERCIALLY VIABLE SOLUTIONS

- Extensive experience with field experiments
- Commercial function and research function are closely integrated
- Allows Valagro to fast-track product candidates with the best chance of attaining commercial viability

THANK YOU
FOR YOUR ATTENTION

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