



**CATRIN**

Czech Advanced  
Technology and Research  
Institute

# Plant phenomics – ways to use it to address research questions

Lukáš Spíchal

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Palacký University Olomouc, Czech Republic

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Who we are (<https://www.catrin.com/research-groups/phenotyping/>)

~ 9 FTEs

- Spíchal Lukáš
- Aucique Perez Carlos Eduardo
- De Diego Sánchez Nuria
- Hybenová Andrea
- Jasso Robles Francisco Ignacio
- Klimeš Pavel
- Mazura Pavel
- Nosková Jana
- Ulbrichová Markéta
- Nisler Jaroslav

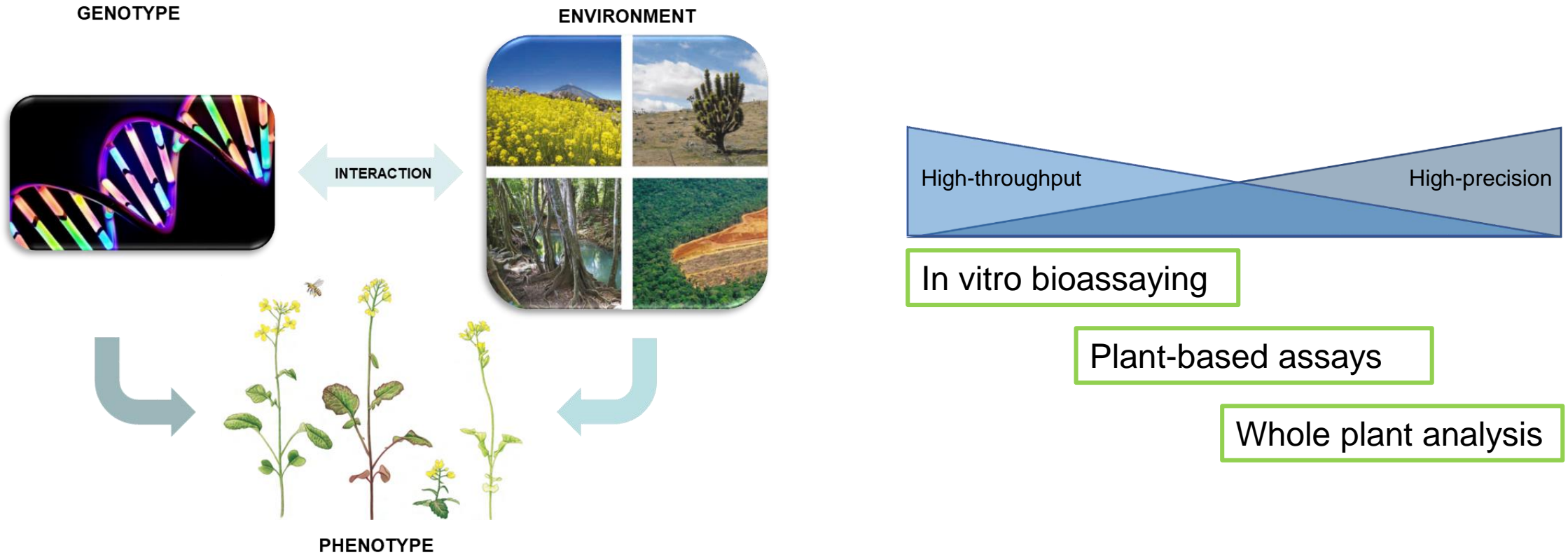
**By professions:**

- Plant biologists
- Molecular biologists
- Plant physiologists / agronomists
- Biochemists
- Analytical chemists
- Organic chemists

**By needs also:**

- HW/SW developers
- Data analysts

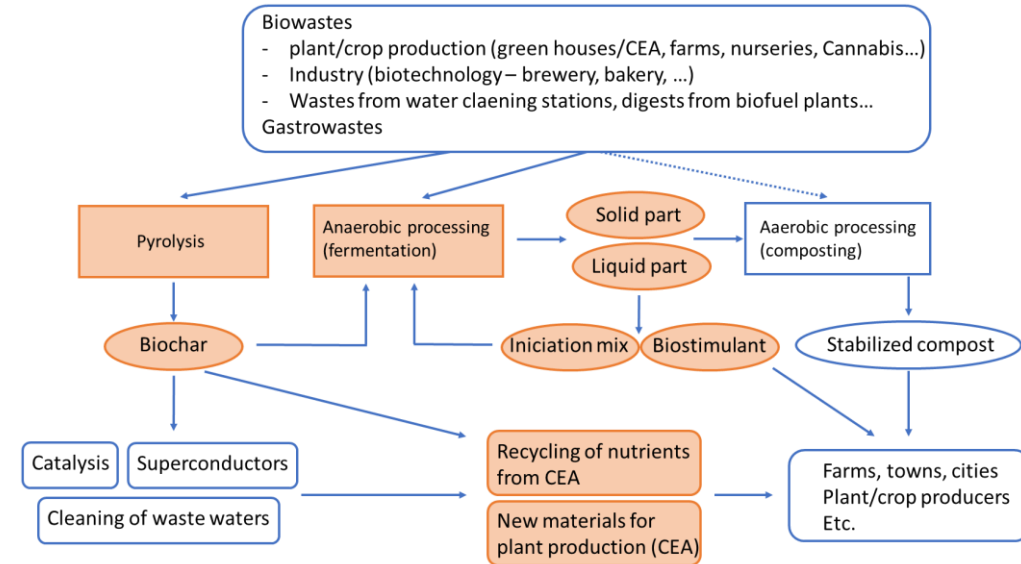
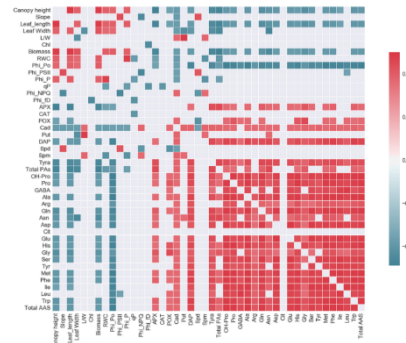
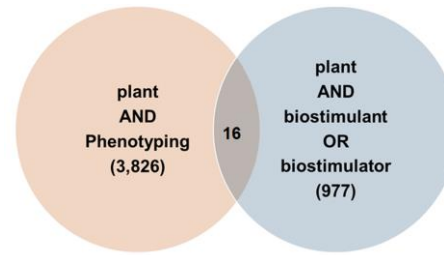
# Monitoring of environmental interactions of plants

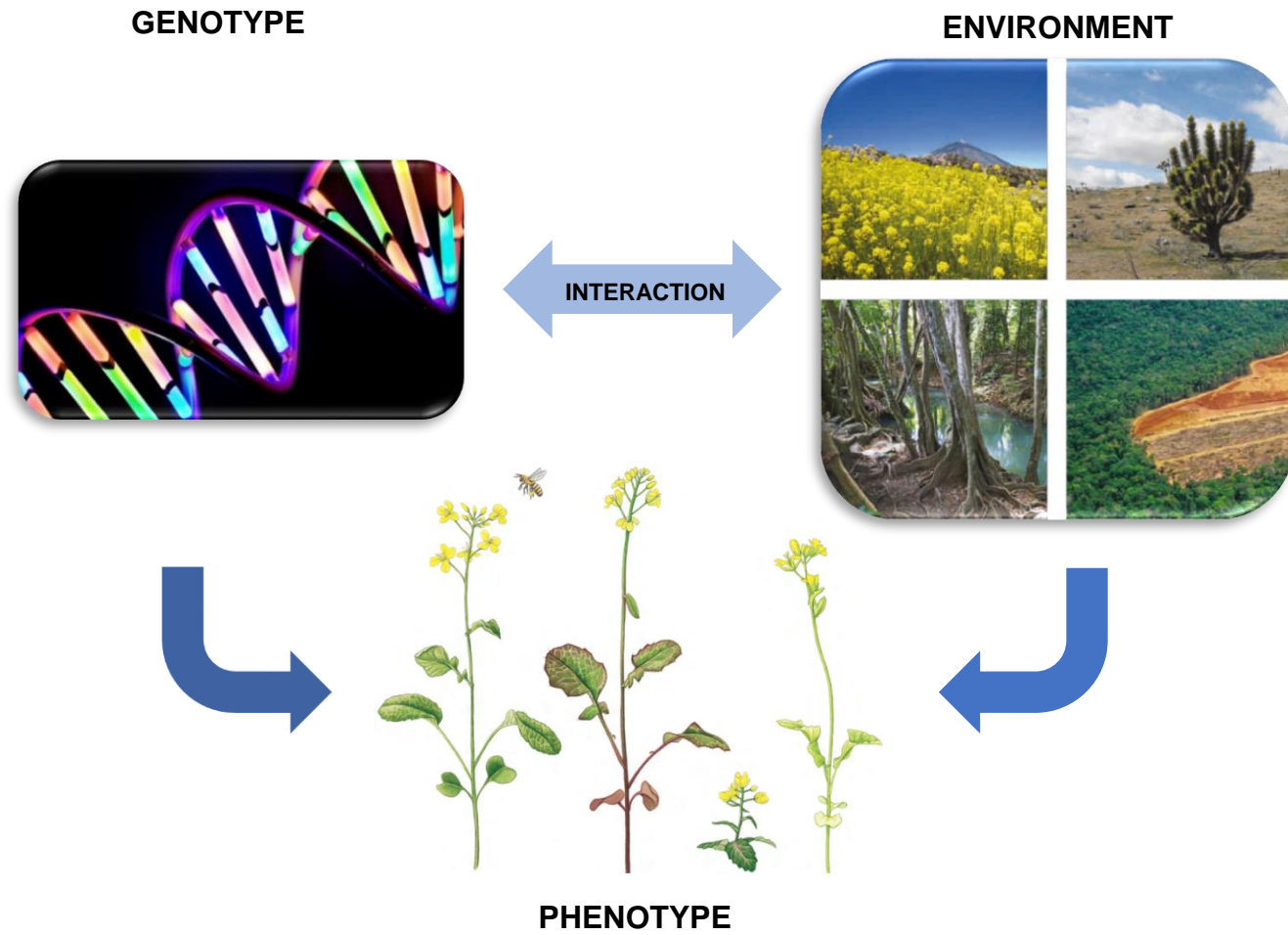


Key words: Phenotype / Non-invasive / Bioassaying / Automation / High-throughput/precision / controlled conditions

## Fields of recent/future interest

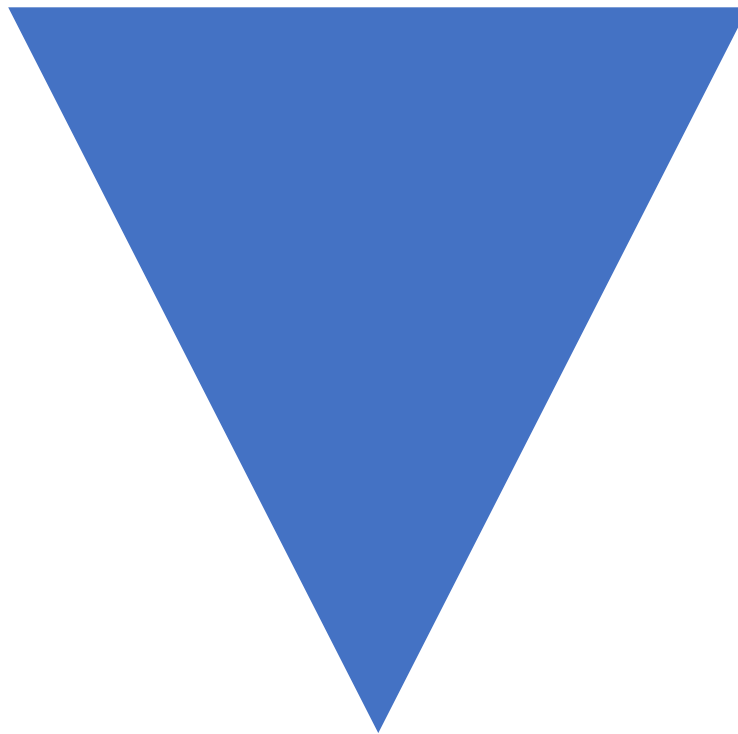
- Plant phenotyping
  - HTS/phenotype-based bioassaying
  - Integration of omic- approaches
  - Affordable/low cost phenotyping
- Biostimulant research
- Polyamine metabolism/transport
- Biowaste valorization
- CEA / biosensors



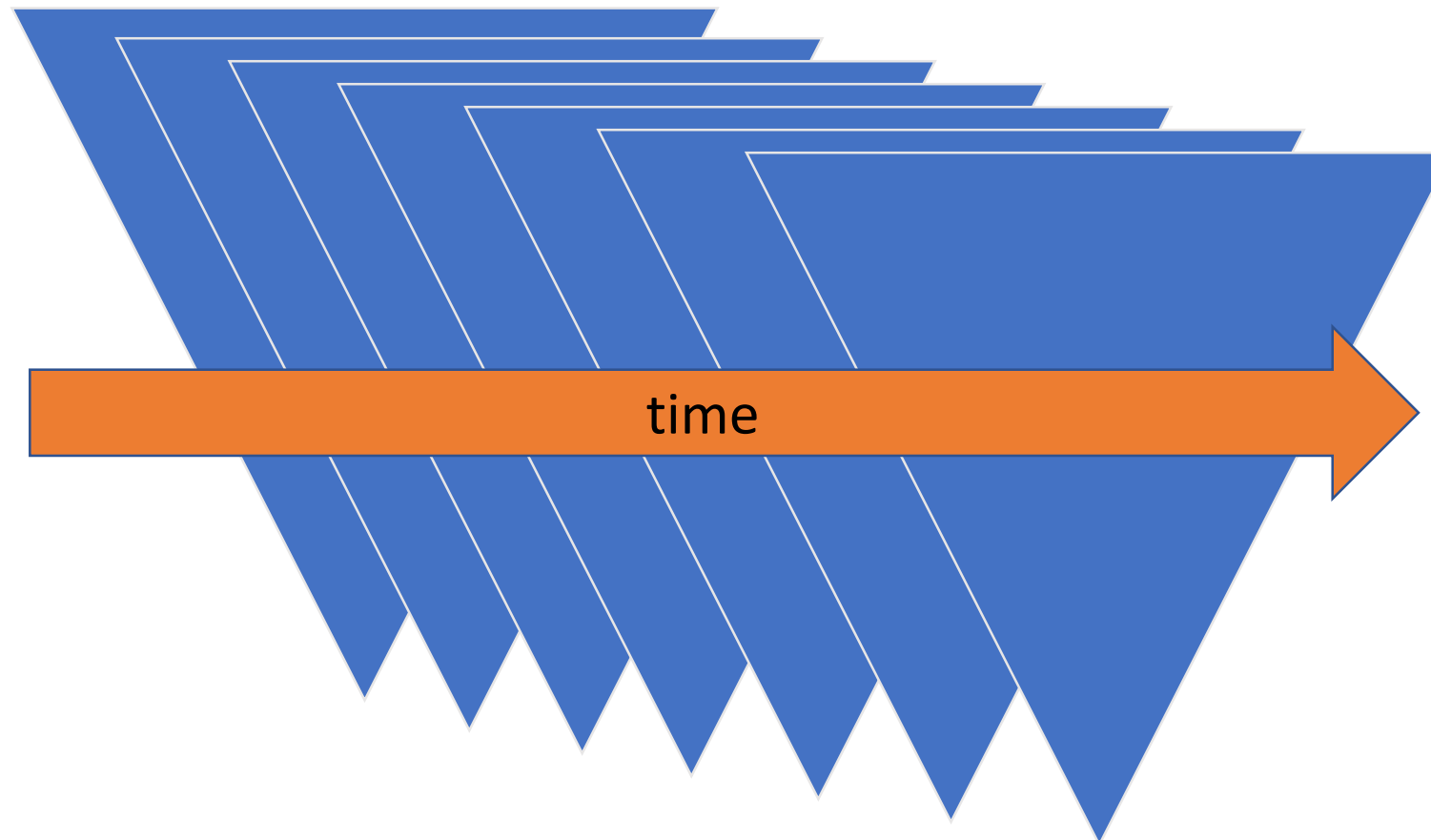


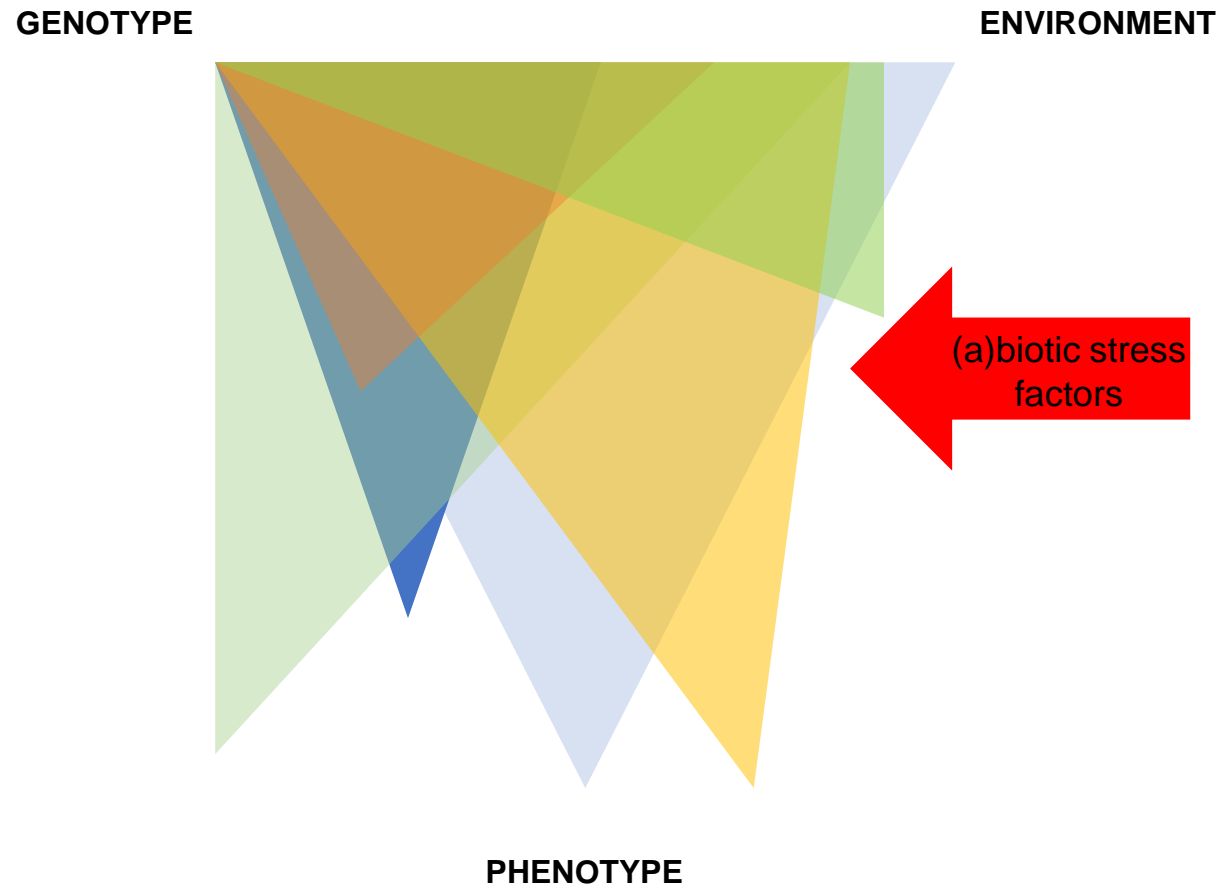
**GENOTYPE**

**ENVIRONMENT**

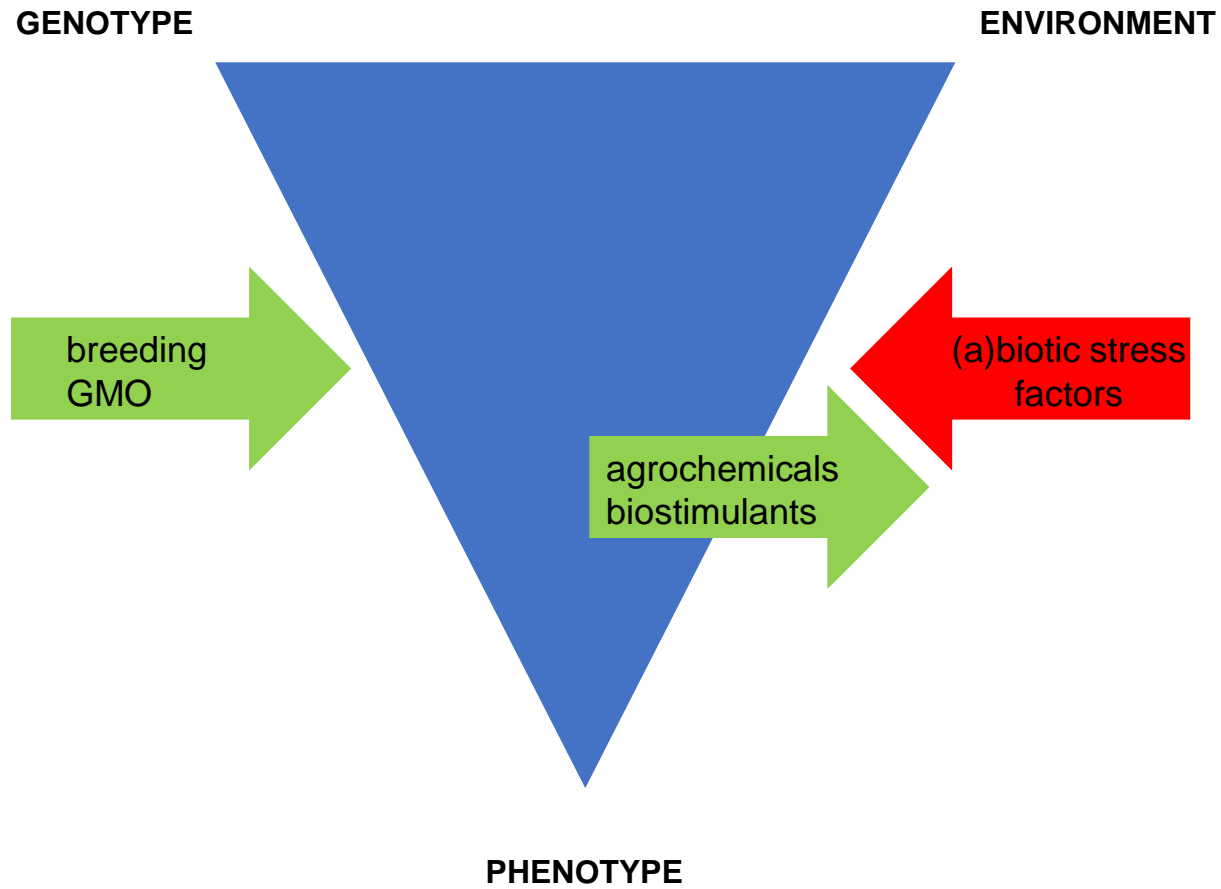


**PHENOTYPE**







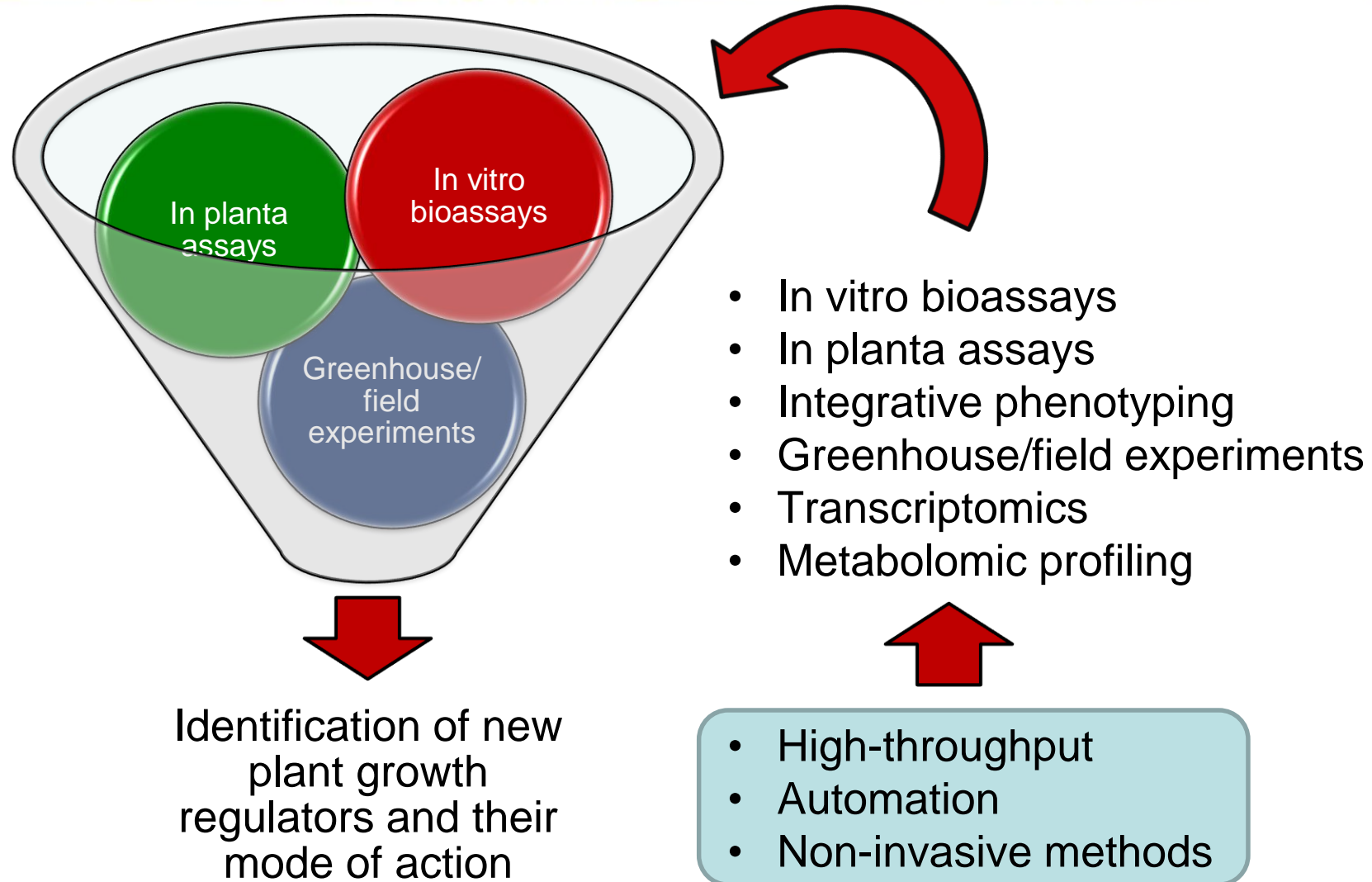


**PHENOTYPE  
PLACTICITY**

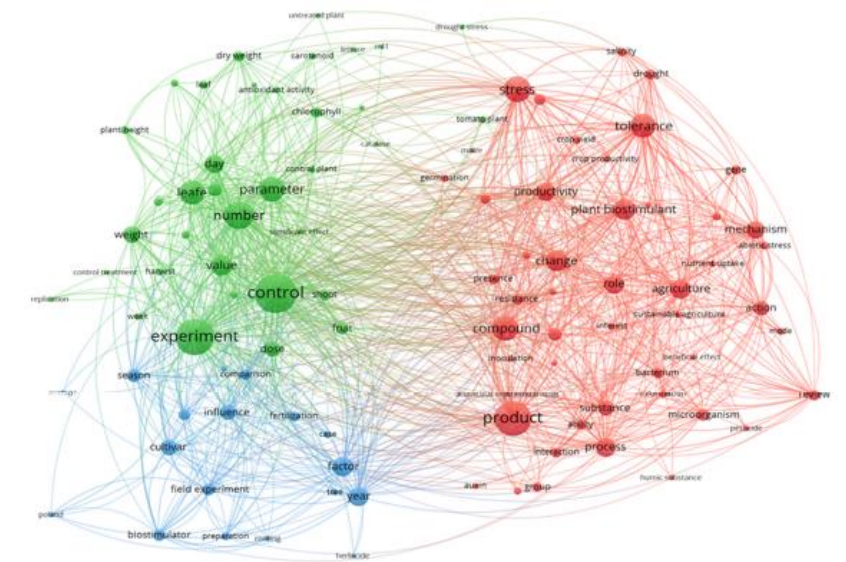
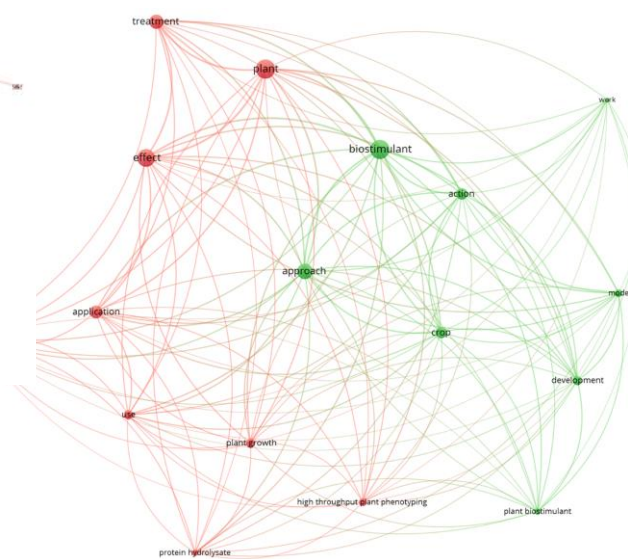
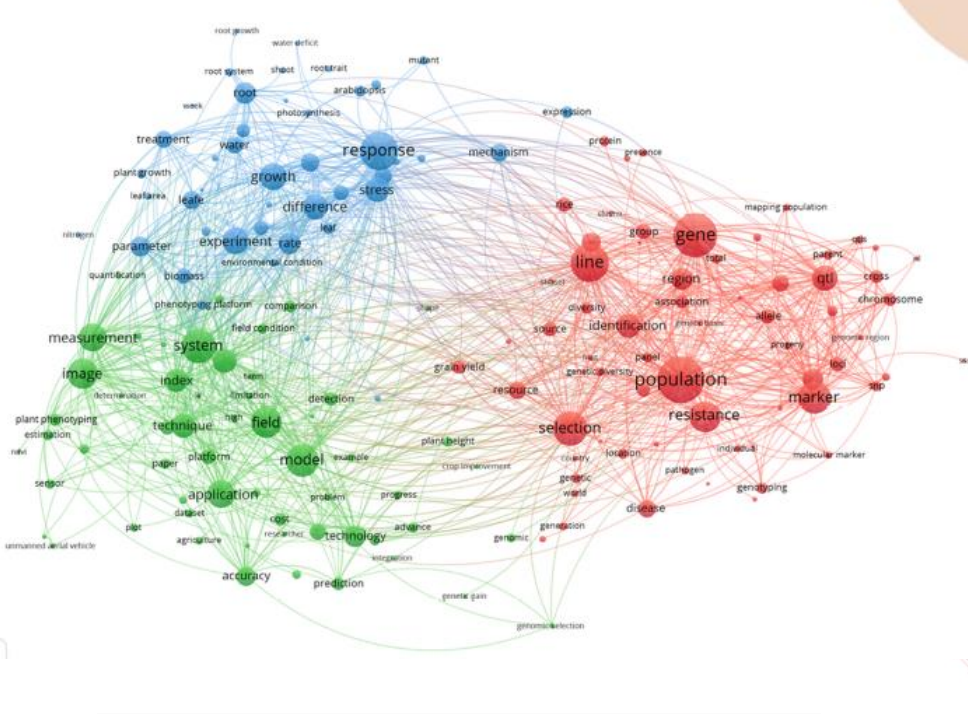
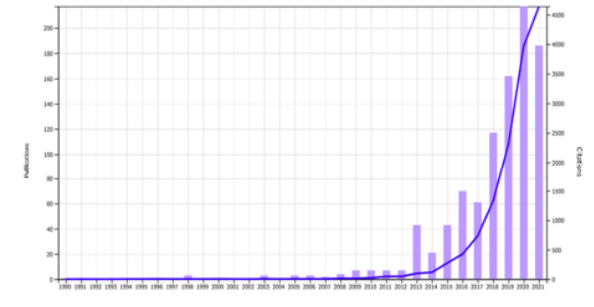
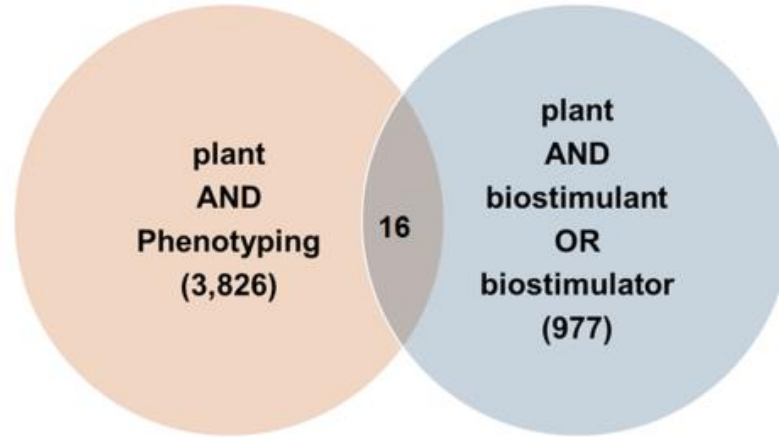
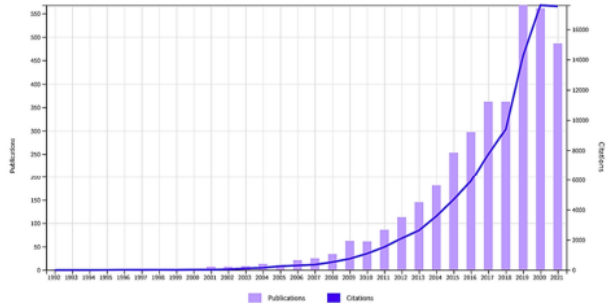
Palacký University  
Olomouc

EDUCATION  
PARTNER

# Automated bioassaying and phenotyping pipe-line



# Evolution of Phenotyping (and related domains)



**Plant phenotyping:** Plant breeding (QTLs) → New non-invasive systems to phenotype plants → data analysis and deep learning

**Biostimulants:** Testing products in simple assays → new parameters studied under controlled conditions or stress → mechanism of action in plants under salt and drought conditions

**Plant phenotyping and biostimulants:** New **approaches for testing** crop development induced by biostimulants → type of treatment and their mode of action

# Automated bioassaying and phenotyping pipe-line

## Source

genotypes

compounds

extracts

microbes

VCs

collections

libraries

batches

series

...

## Application

seed treatment

foliar

drenching

## Assay

*in vitro* bioassays

*in planta* assays

Hormonal response

Emergence

Seed germination

Early development

Shoot growth response

Growth and physiology

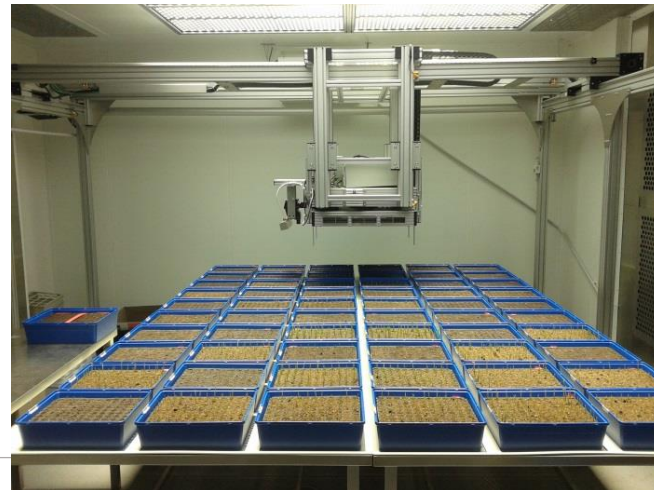
## Conditions

normal

abiotic stress

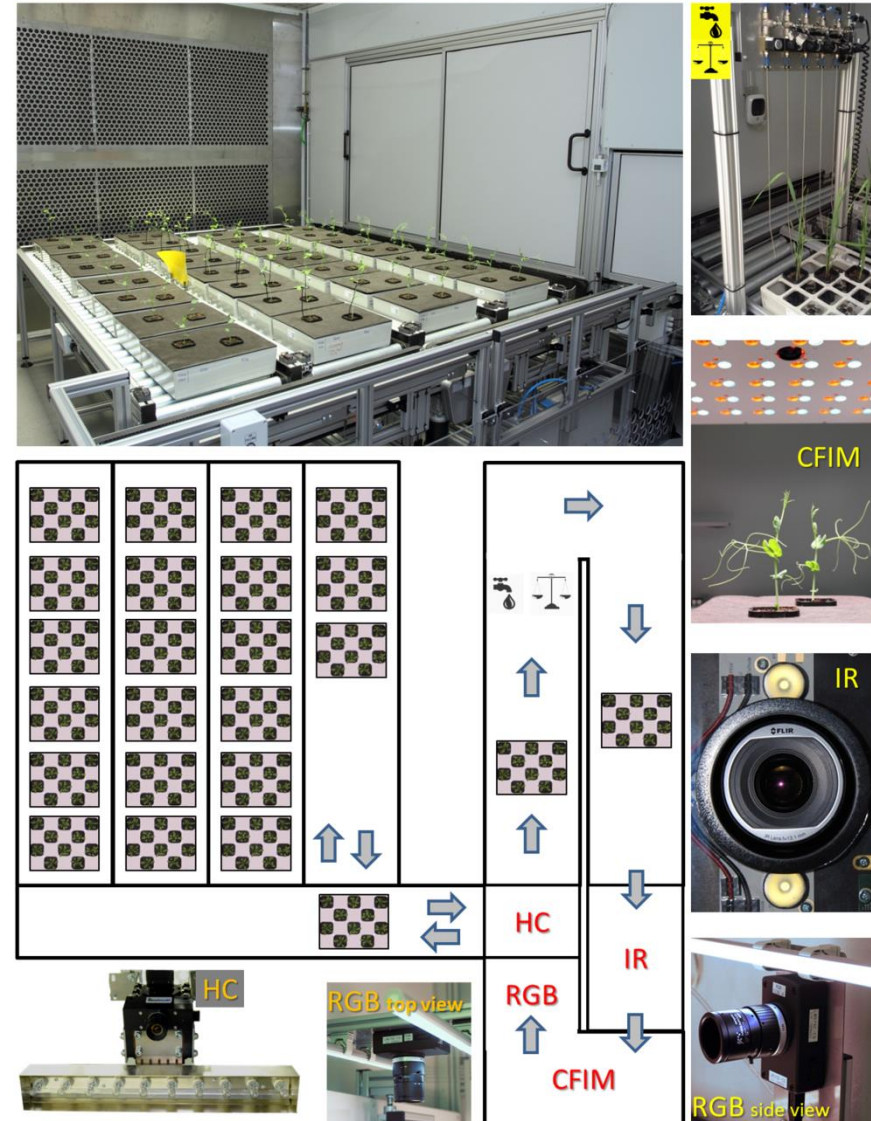
biotic stress

- Controlled conditions
- plant growth sensor (RGB top view high-resolution camera with homogenous LED lightning)
- sensors of physiological responses:
  - FluorCam unit – Chl flurescence kinetic analysis
  - hyperspectral unit (VIS 380-1000 nm)
- capacity: 7.5 square metres (528 culture multiwell plates, 64 trays, 1280 standardized Arabidopsis pots)



- Controlled conditions
- three RGB cameras, FluorCam, thermoimaging, hyperspectral imaging (1000-2500 nm), acclimation cabinet, automatized pot weighing and watering
- capacity: 640 plants for top-view experiments, 64-32 plants for three-views experiments

Humplík JF, Lazar D, Husičková A, Spíchal L (2015) Automated phenotyping of plant shoots using imaging methods for analysis of plant stress responses – a review. *Plant Methods*, 11:29.





# Automated bioassaying and phenotyping pipe-line

## We test

- genotypes
- Compound libraries
- Extracts
- Compounds/mixtures
- PGPR
- microbes
- VCs
- ...
- Commercial products
  - Series
  - Libraries
  - Batches
  - ...
- Way of application
  - Seed treatment
  - drench
  - foliar

## In vitro bioassays

- Hormone signaling response
- Seed germination
- Shoot growth response

## In planta assays

- Crop emergence
- Early development
- Shoot growth and physiology

## Greenhouse/field experiments

- Yield parameters
- Plant physiology

## Metabolomic profiling

- Amino acids
- Plant hormones
- Phenolic compounds
- Polyamines

## Transcriptomics



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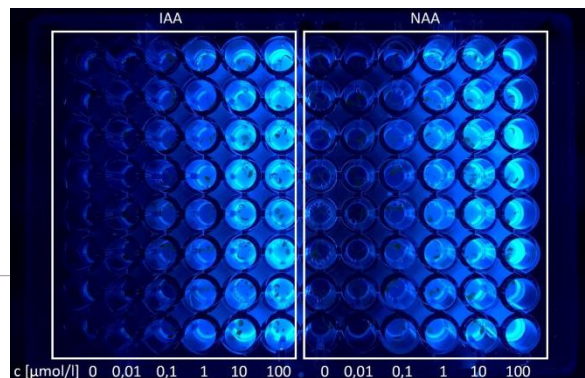
### Characteristics:

- Fast (in days)
- High-throughput (hundreds of variants)
  - Time series design (kinetic)
  - High number of combinations
  - High number of replicates
  - Population behavior
  - Statistical approaches

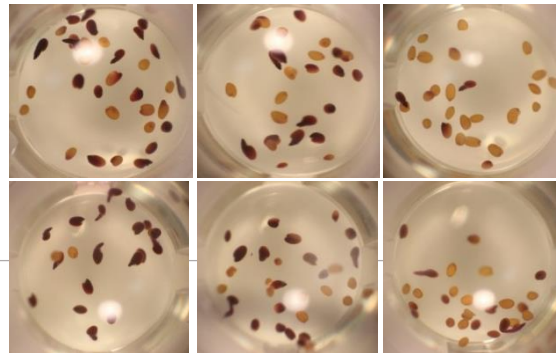
### Using multiwell plates

- 6-, 24-, 48-, 96-well plates
- Non-invasive simple readout

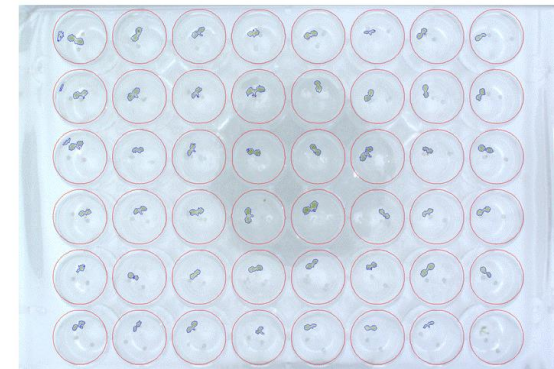
Hormone signaling response



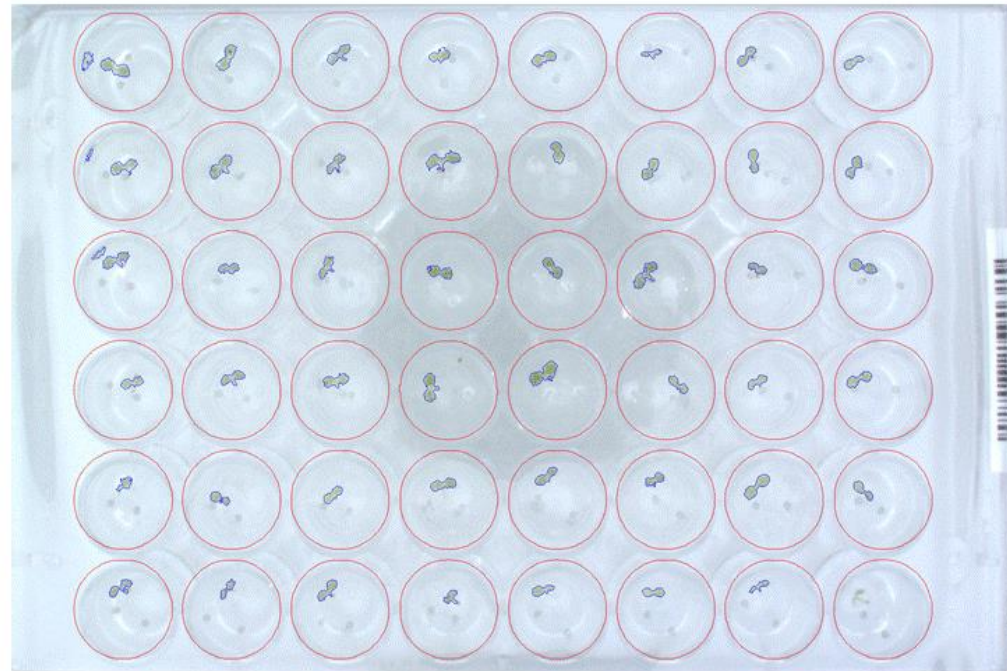
Seed germination



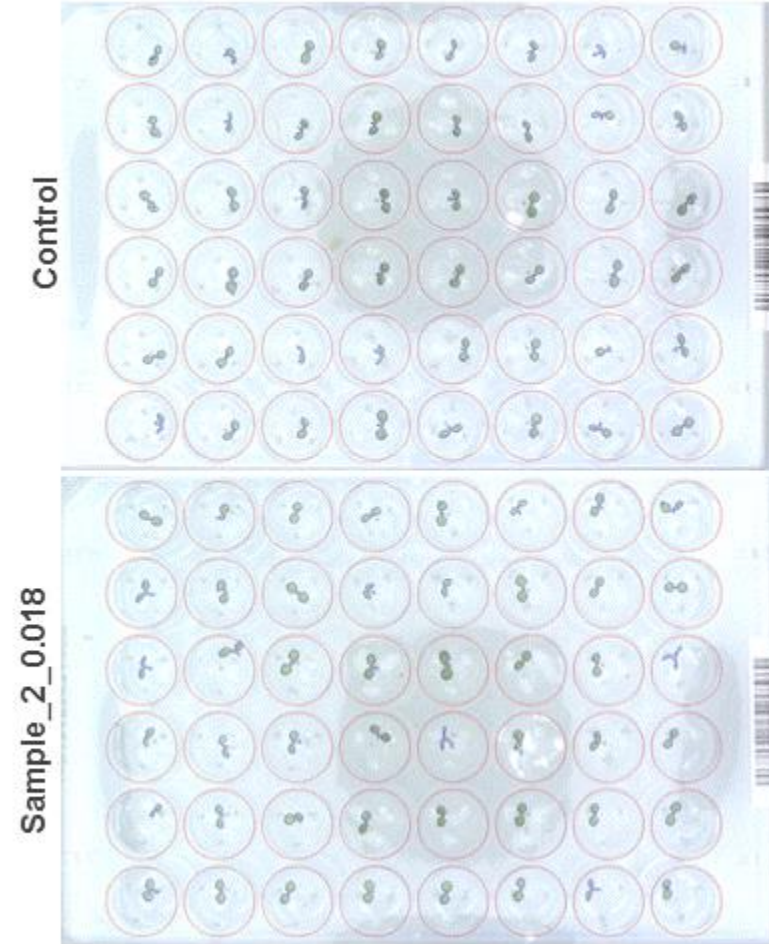
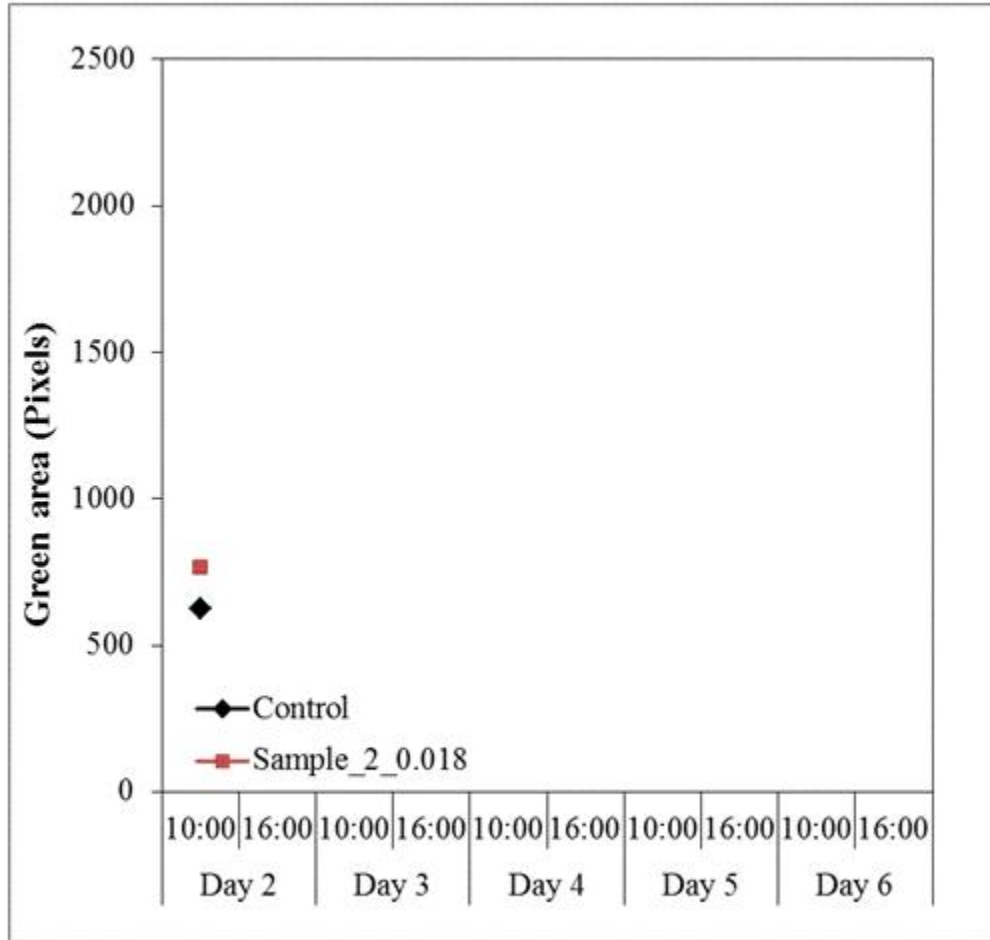
Shoot growth response



# In vitro bioassays – Shoot growth response



# In vitro bioassays – Shoot growth response



- Analyses of effect on shoot area of *Arabidopsis*
  - Stimulation/Inhibition of shoot growth
  - normal conditions / Interaction with stress conditions
    - Salt, temperature, nutrition, drought, chemicals, pathogen response

**METHODS ARTICLE**

Front. Plant Sci., 04 October 2017 | <https://doi.org/10.3389/fpls.2017.01702>

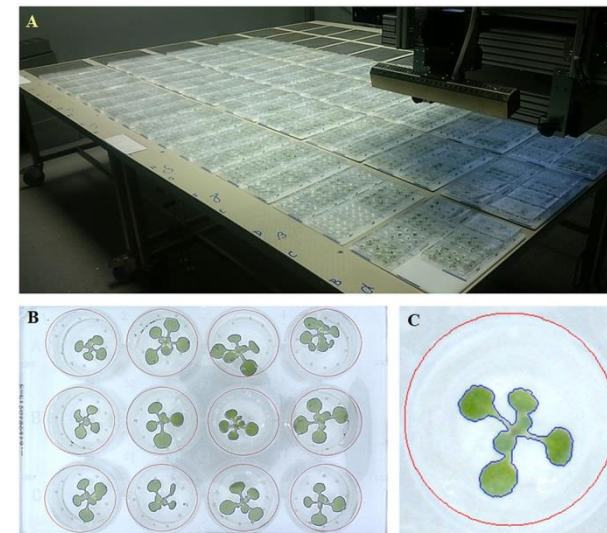


## An Automated Method for High-Throughput Screening of *Arabidopsis* Rosette Growth in Multi-Well Plates and Its Validation in Stress Conditions

Nuria De Diego<sup>1</sup>, Tomáš Fůrst<sup>1</sup>, Jan F. Humplík<sup>1,2</sup>, Lydia Ugena<sup>1</sup>, Kateřina Podlešáková<sup>1</sup> and Lukáš Spíchal<sup>1\*</sup>

<sup>1</sup>Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czechia

<sup>2</sup>Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Czech Academy of Sciences, Olomouc, Czechia



„This approach will allow simultaneous testing of a large number of potentially bioactive compounds in a wide range of concentrations and/or genotypes, under various growth conditions.“ (De Diego et al., 2017)

METHODS ARTICLE

Front. Plant Sci., 04 October 2017 | <https://doi.org/10.3389/fpls.2017.01702>



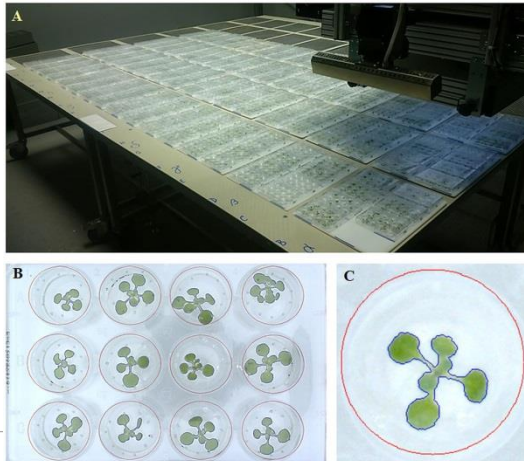
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# Characterization of Biostimulant Mode of Action Using Novel Multi-Trait High-Throughput Screening of *Arabidopsis* Germination and Rosette Growth

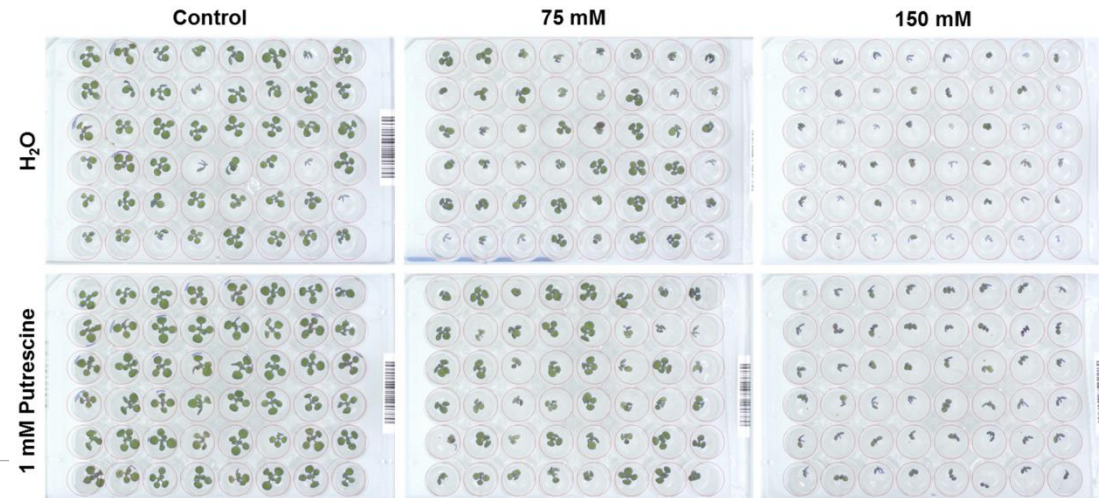
Lydia Ugena<sup>1†</sup>, Adéla Hýlová<sup>1†</sup>, Kateřina Podlešáková<sup>1</sup>, Jan F. Humplik<sup>1,2</sup>, Karel Doležal<sup>1</sup>, Nuria De Diego<sup>1\*</sup> and Lukáš Spíchal<sup>1</sup>

6-, 12-, 24-well plates



De Diego et al., 2017

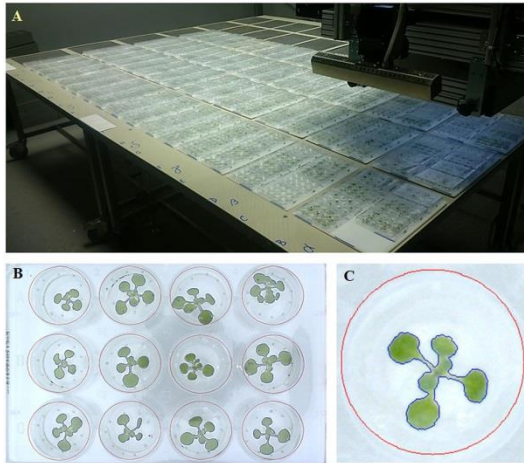
48-well plates



Ugena et al., 2018

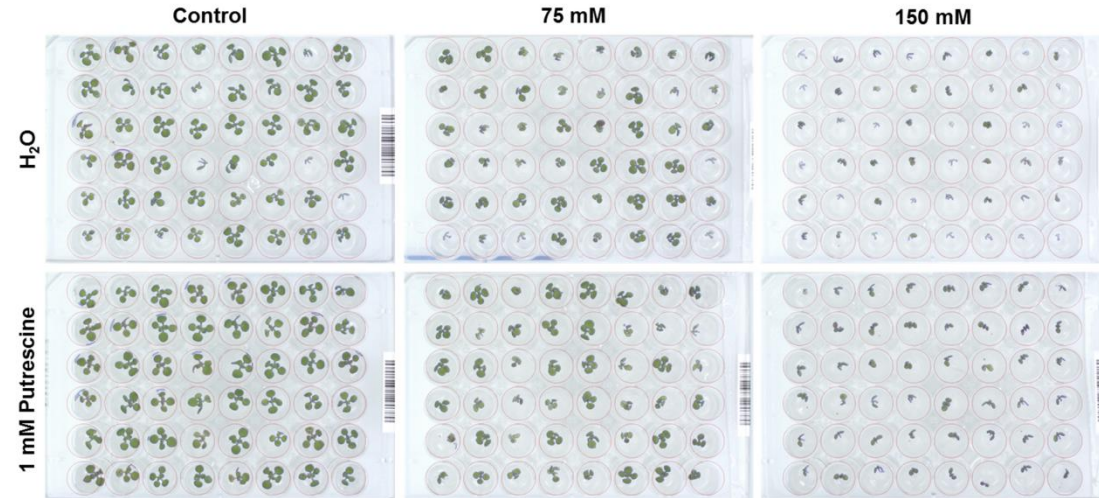
# In vitro bioassays – Shoot growth response

6-, 12-, 24-well plates



De Diego et al., 2017

48-well plates



Ugena et al., 2018

Type of well plate	No. plants	Replicates	Platform capacity	Total plants	No. variants	Assay duration
6-Well Plates	6	3	480 Plates	2880	160	14 days
12-Well Plates	12	2		5760	240	9 days
24-Well plates	24	1		11520	480	9 days

48-well plates

528 plates

25344 plants

7 days



# In vitro bioassays – Shoot growth response

48-well plates      528 plates      25344 plants      7 days

1 day / 1 plate



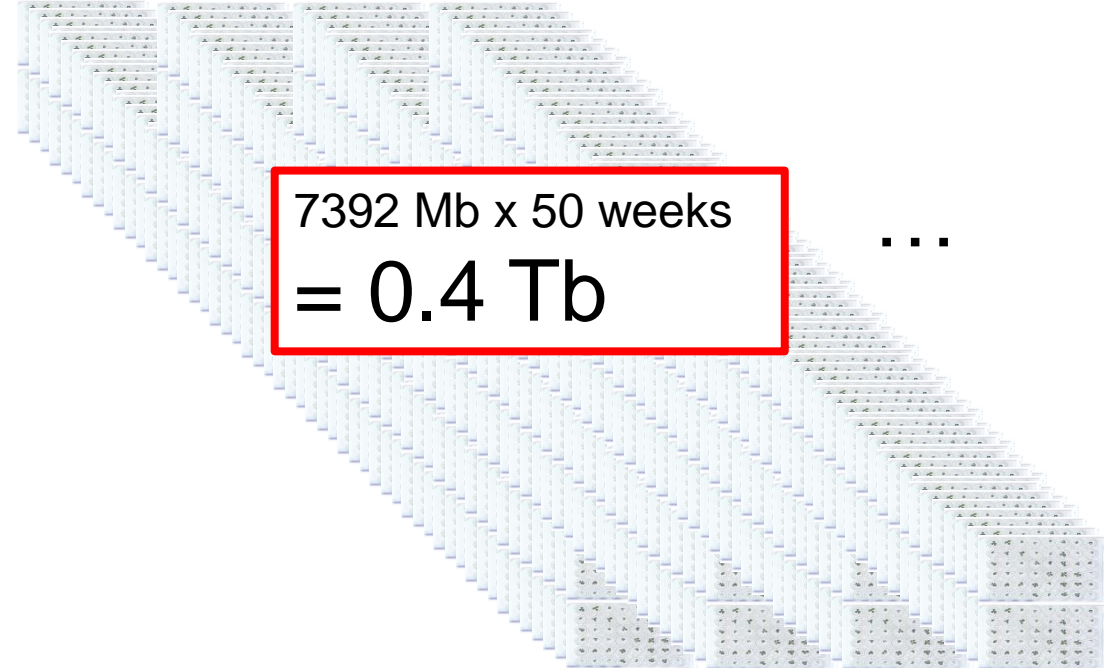
$$2 \times 10 \text{ Mb} = 20 \text{ Mb}$$

1 week / 1 plate



$$2 \times 7 \times 10 \text{ Mb} = 140 \text{ Mb}$$

1 week / 528 plates

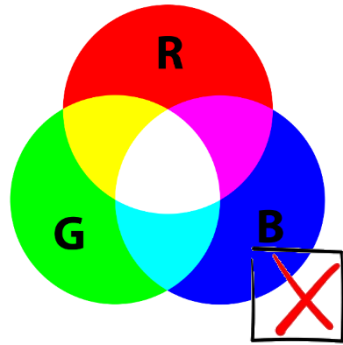


$$2 \times 7 \times 528 \times 10 \text{ Mb} = 7392 \text{ Mb}$$

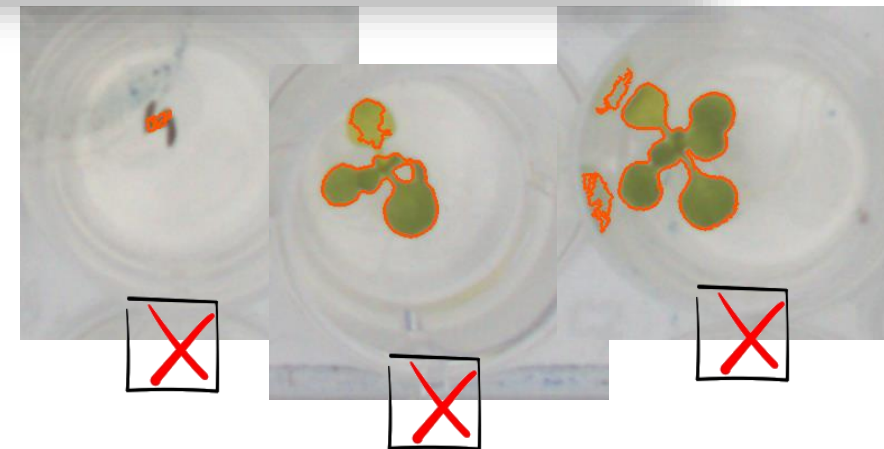
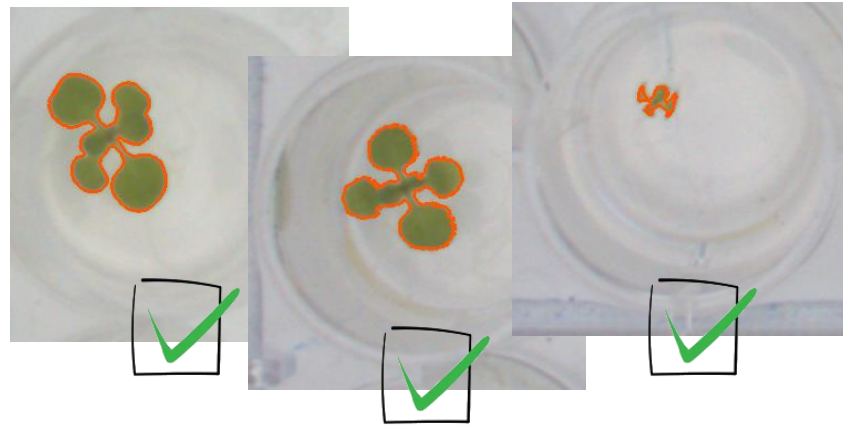
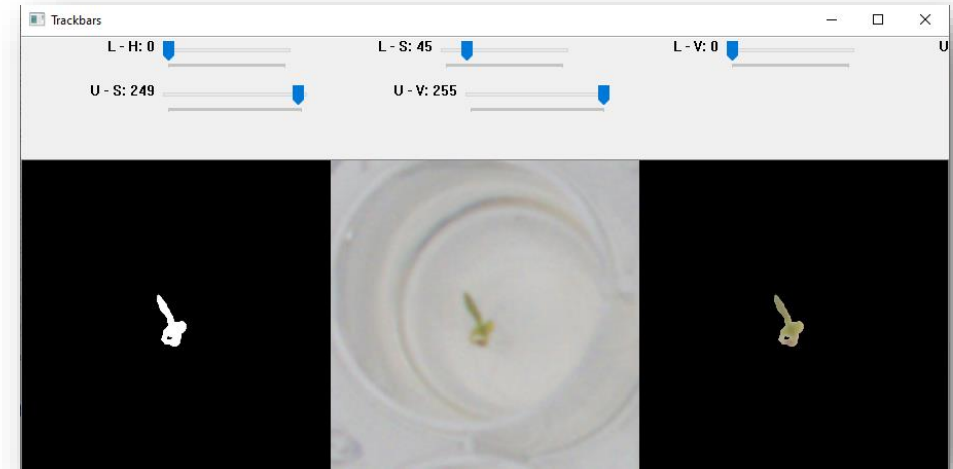
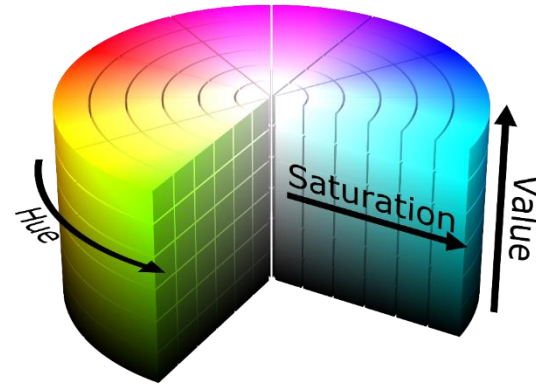
- Python script for evaluation of plant properties in multiwell plates

- Colour based analysis

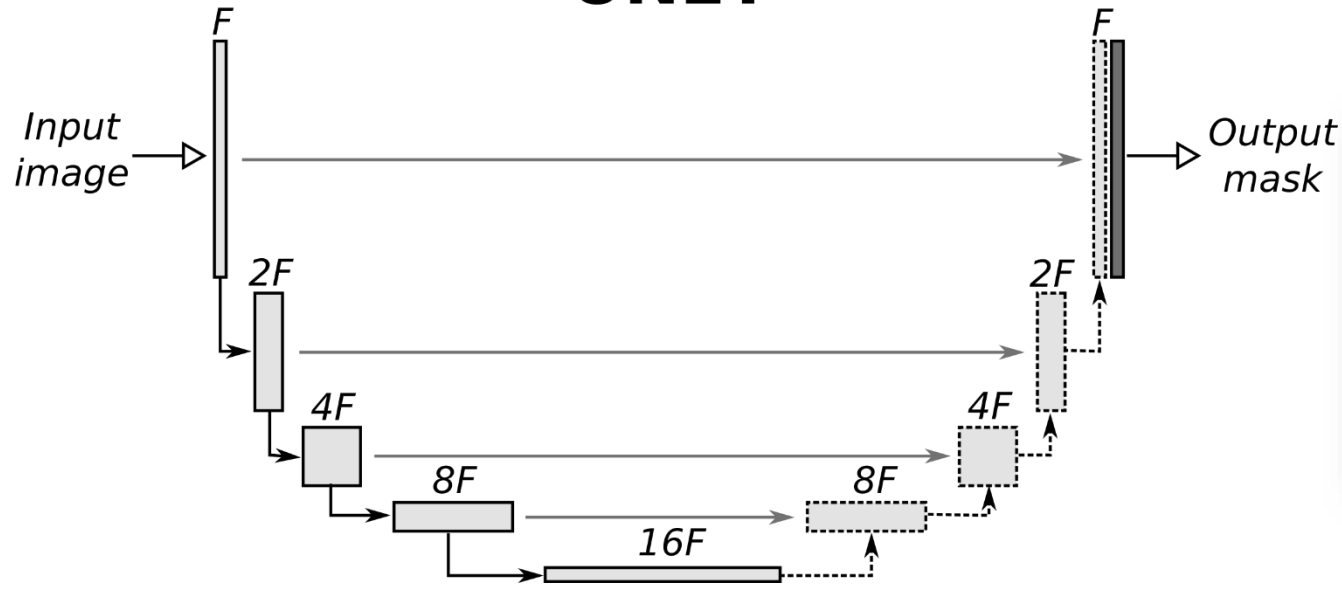
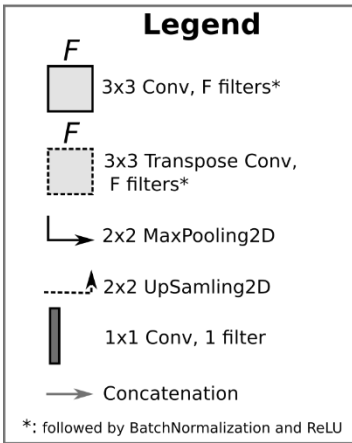
- RGB



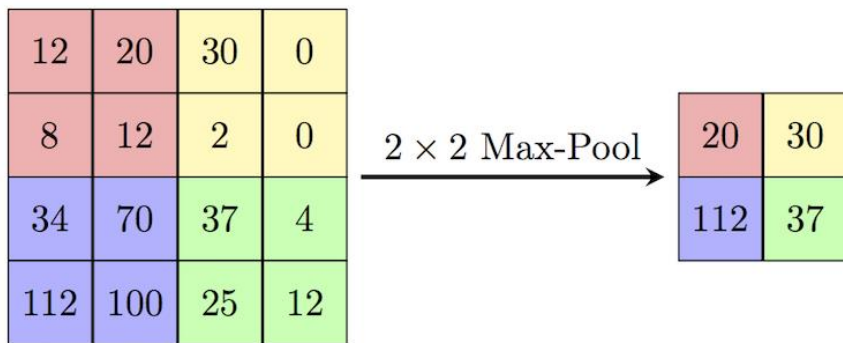
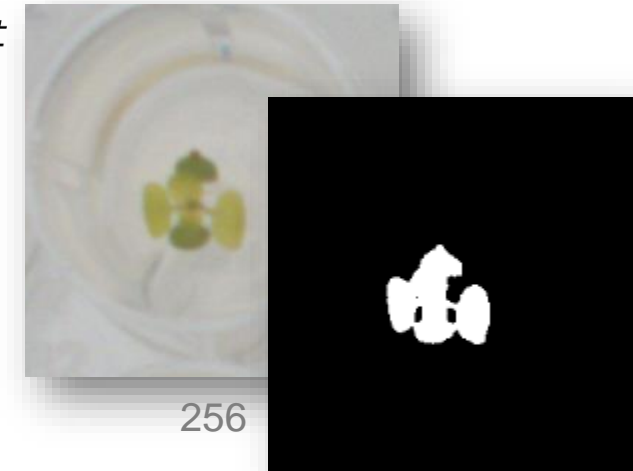
- HSV



## UNET

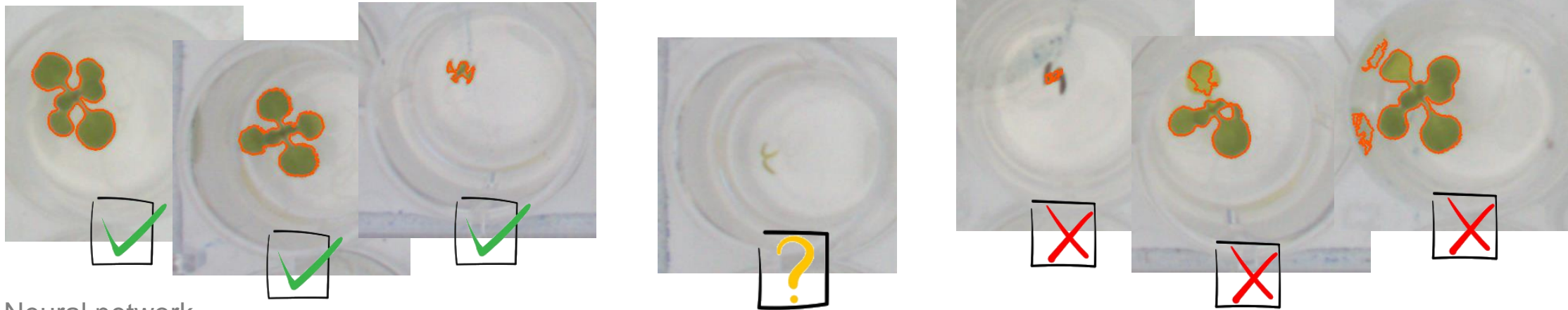


- Trained on 500 pictures
- Accuracy 97 %

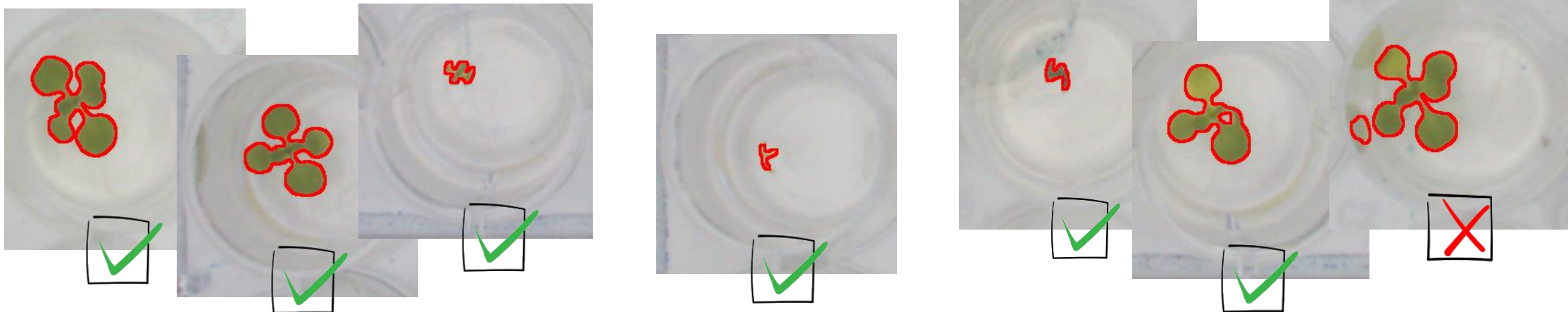


Layer (type)	Output Shape	Param #
input_1 (InputLayer)	[(None, 256, 256, 3)]	0
.....		
conv2d_18 (Conv2D)	(None, 256, 256, 1)	17
-----		
Total params: 1,941,105		
Trainable params: 1,941,105		
Non-trainable params: 0		

- Color based analysis

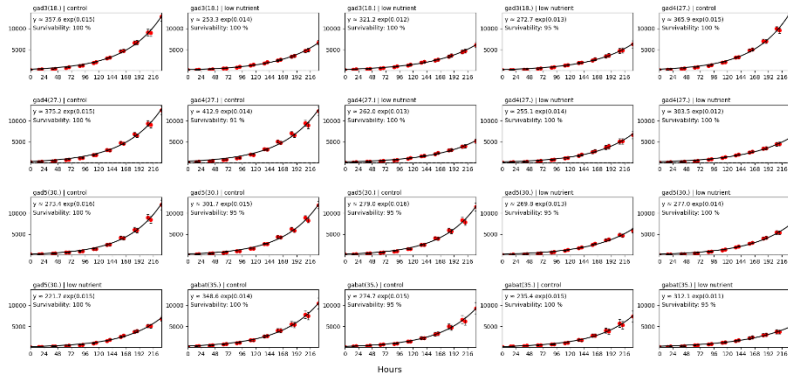


- Neural network

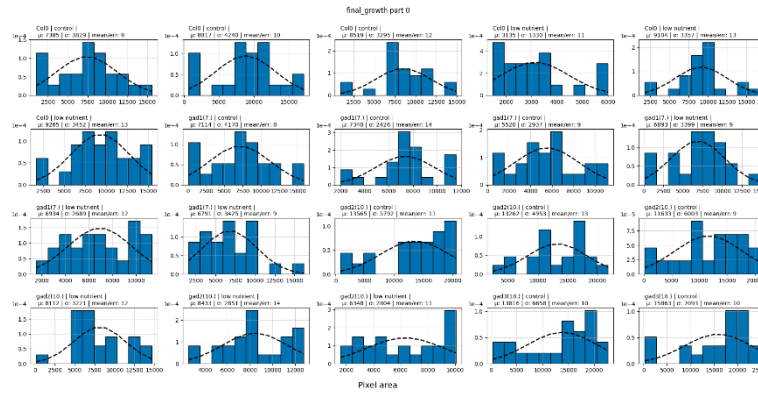


- Parameters: Area, Diameter, RGB ...

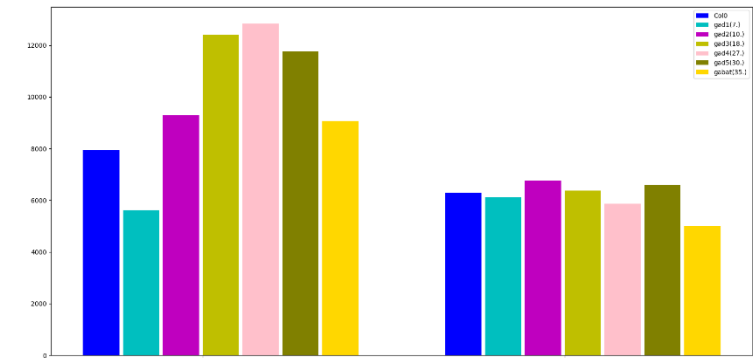
**Over time plot for individual plates**



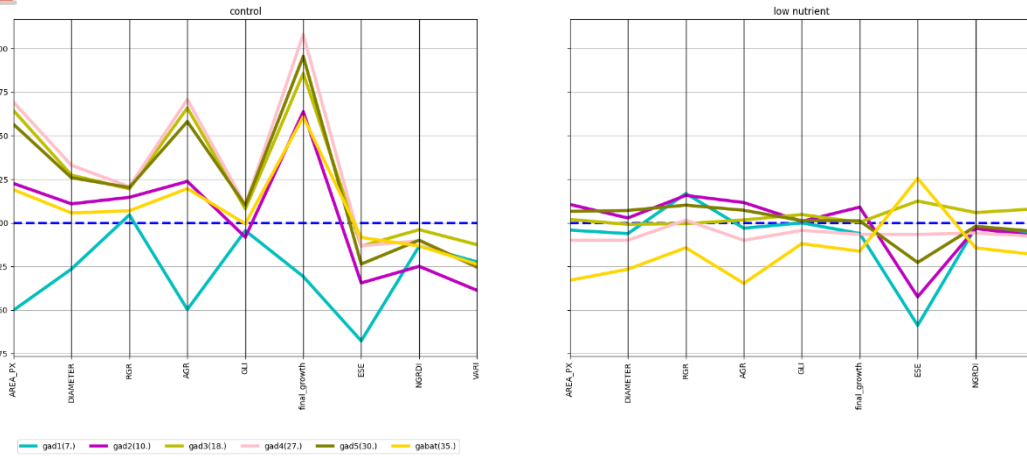
**Distribution of param. values within a plate**



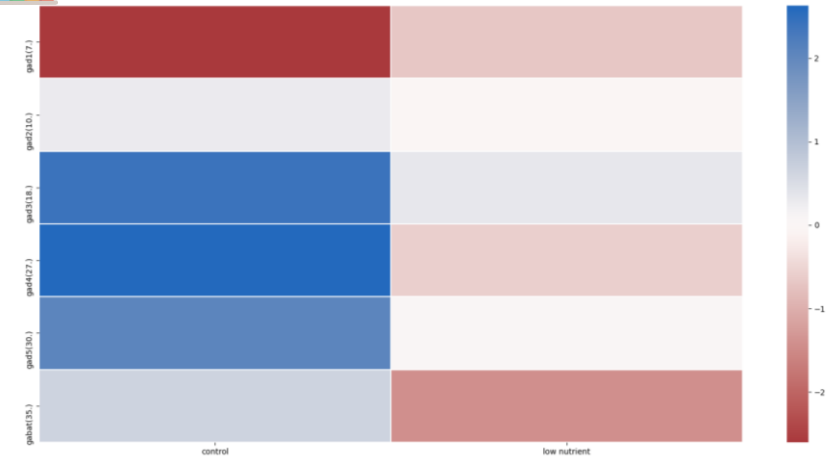
**Plot at the end of the experiment**



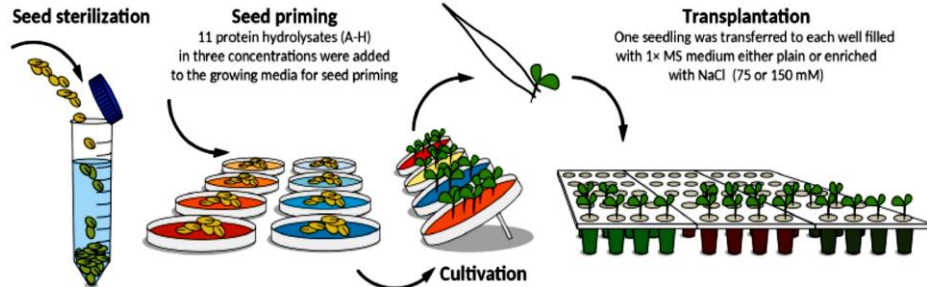
**Parallel coordination plot for all parameters together**



**PBC index plot**



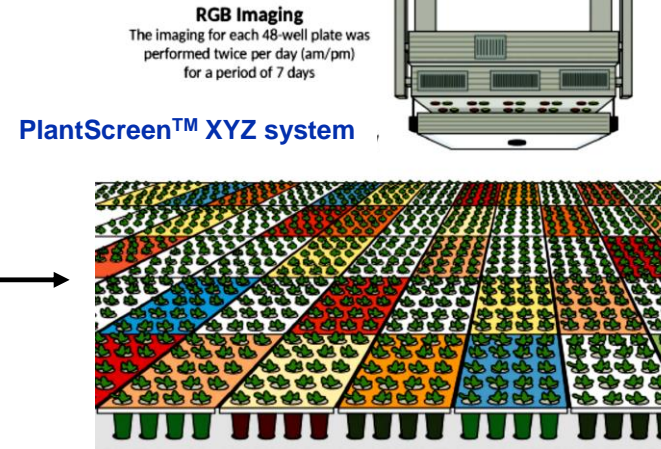
## Material preparation



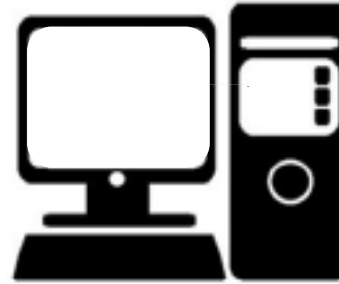
Synthetic compounds  
Adjuvants  
Biostimulants

WIDE RANGE OF  
CONCENTRATIONS

## High-Throughput phenotyping



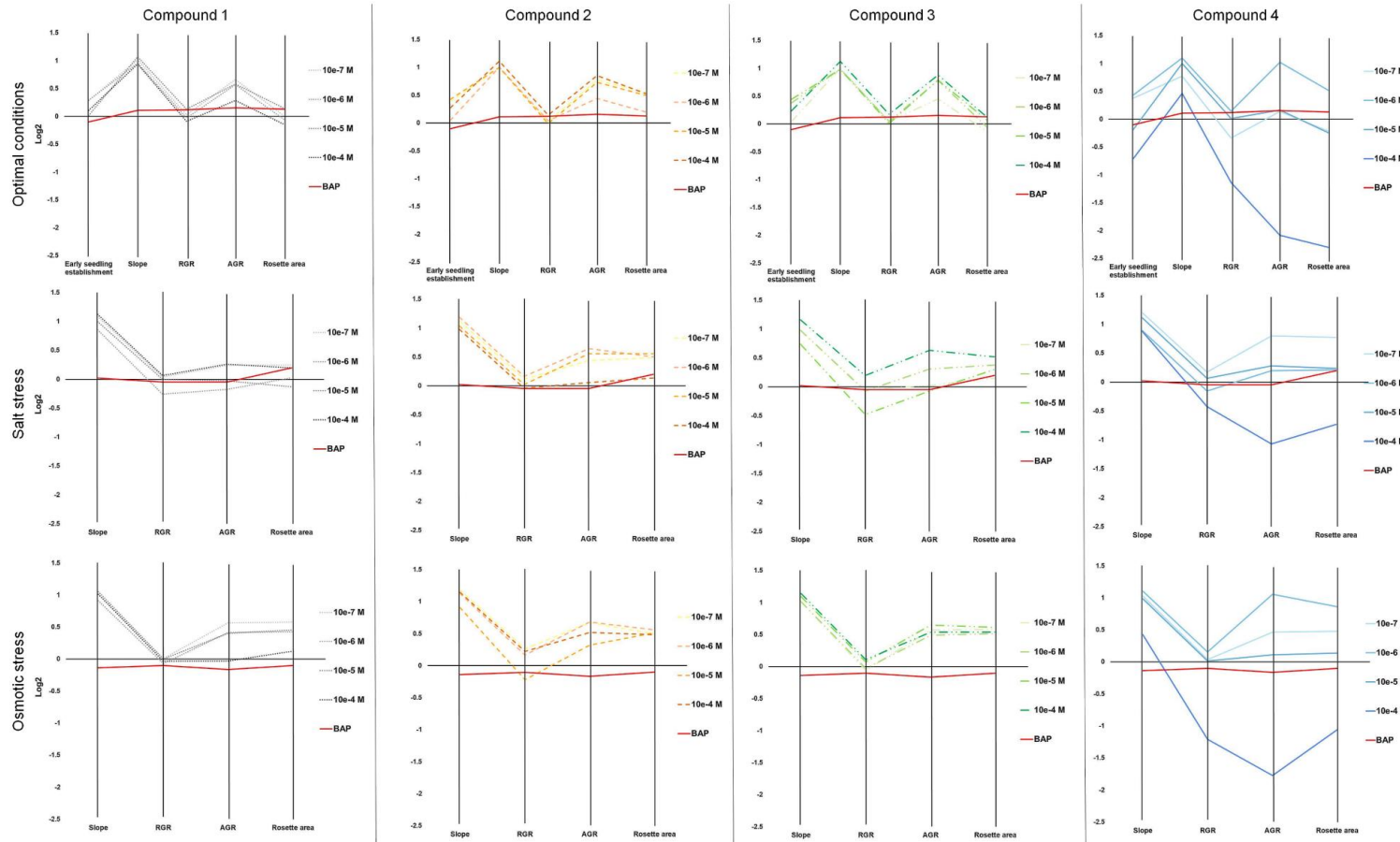
## Data analysis



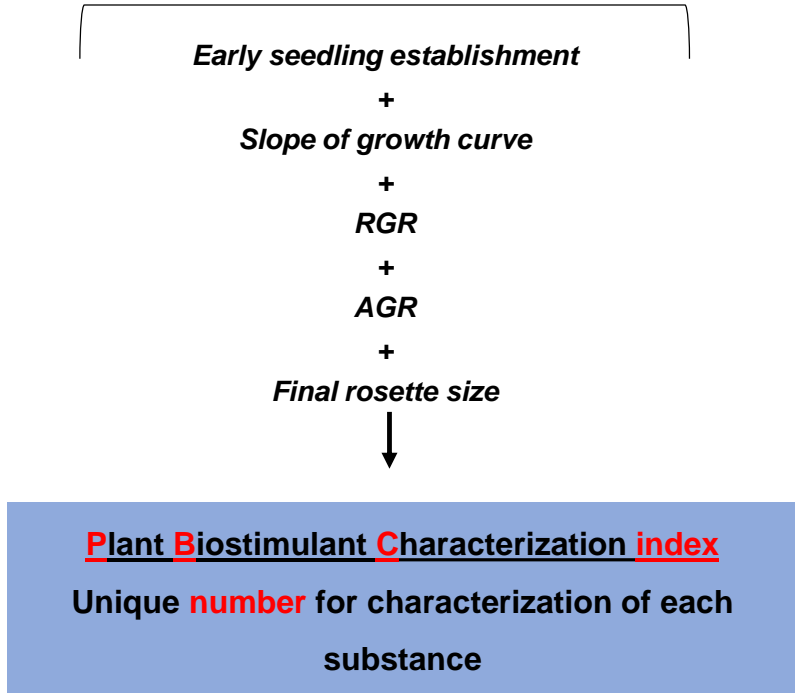
- *Early seedling establishment*
- *Slope of growth curve*
- *RGR*
- *AGR*
- *Final rosette size*
- *Survival*
- *Index color*
- *C.V. (%)*

**PBC index**

## Hormopriming with of $N^9$ -substituted cytokinin derivatives with a fluorinated carbohydrate moiety



Multiple traits  
(Ratio Control/Treated (log<sub>2</sub>))



(Bryksová *et al.* 2020. Front. Plant Sci.)

# Automated bioassaying and phenotyping pipe-line

## We test

- genotypes
- Compound libraries
- Extracts
- Compounds/mixtures
- PGPR
- microbes
- VCs
- ...
- Commercial products
  - Series
  - Libraries
  - Batches
  - ...
- Way of application
  - Seed treatment
  - drench
  - foliar

## In vitro bioassays

- Hormone signaling response
- Seed germination
- Shoot growth response

## In planta assays

- Crop emergence
- Early development
- Shoot growth and physiology

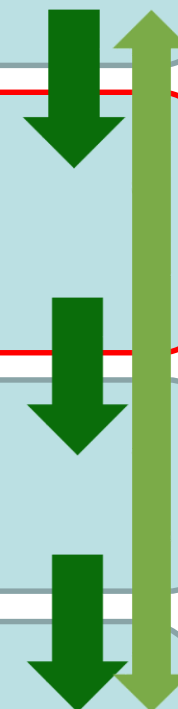
## Greenhouse/field experiments

- Yield parameters
- Plant physiology

## Metabolomic profiling

- Aminoacids
- Plant hormones
- Phenolic compounds
- Polyamines

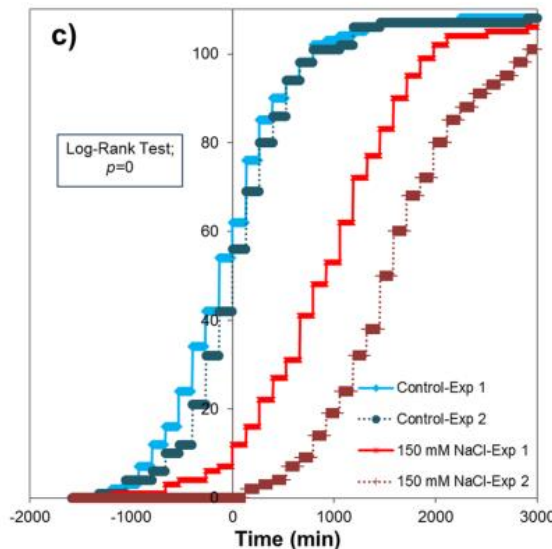
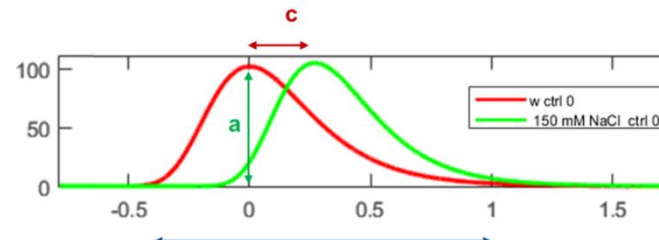
## Transcriptomics

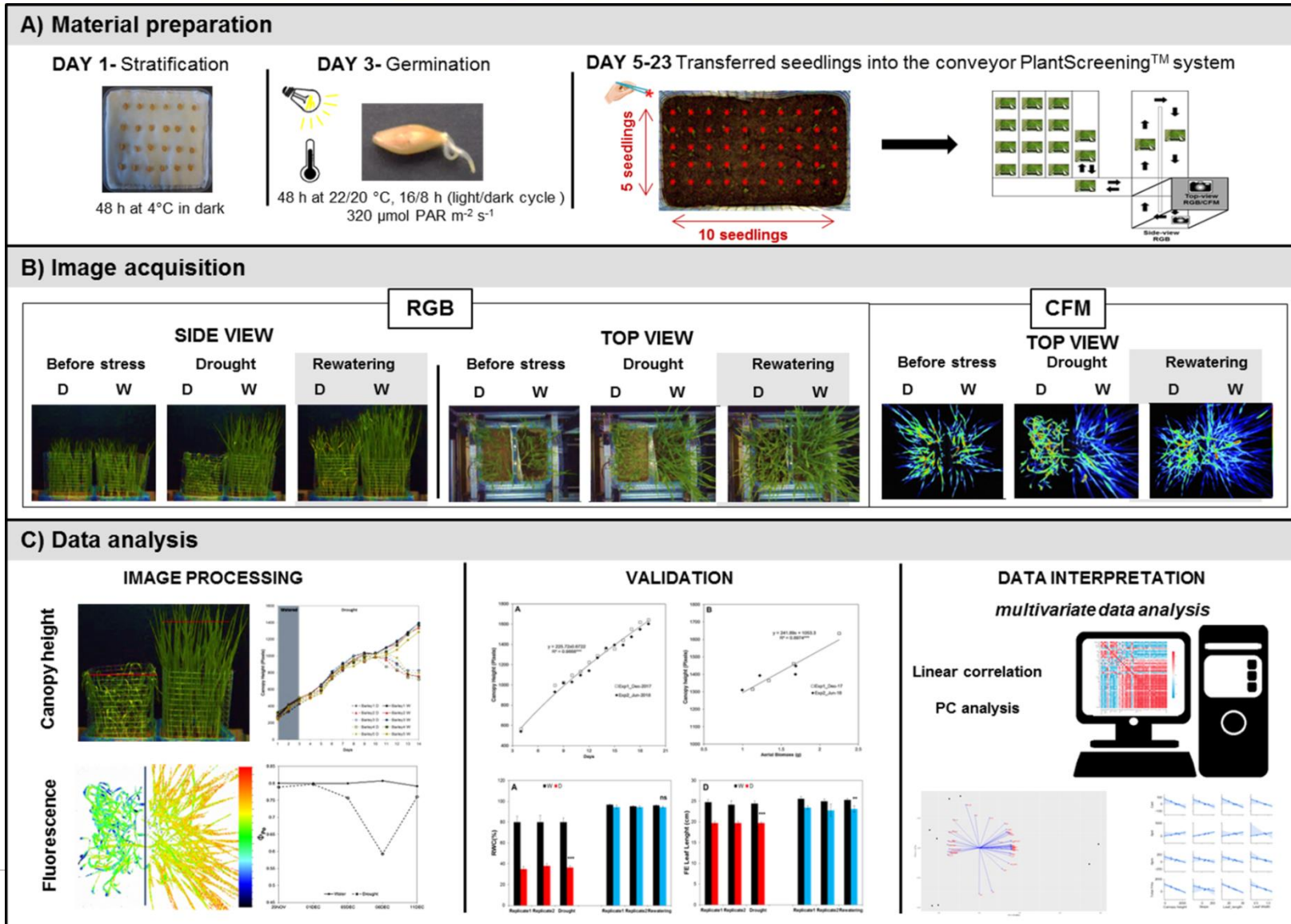




# An automated approach of high-throughput dynamic scoring of crop seedling emergence

- Testing of the emergence of crops (cereals, tomato, rapeseed); different lines and/or after application of various compounds and/or stress conditions (salt, cold, drought)
- Seed treatment
- 64 variants X 110 seeds
- Screening each 2 hours for approx. 7 days

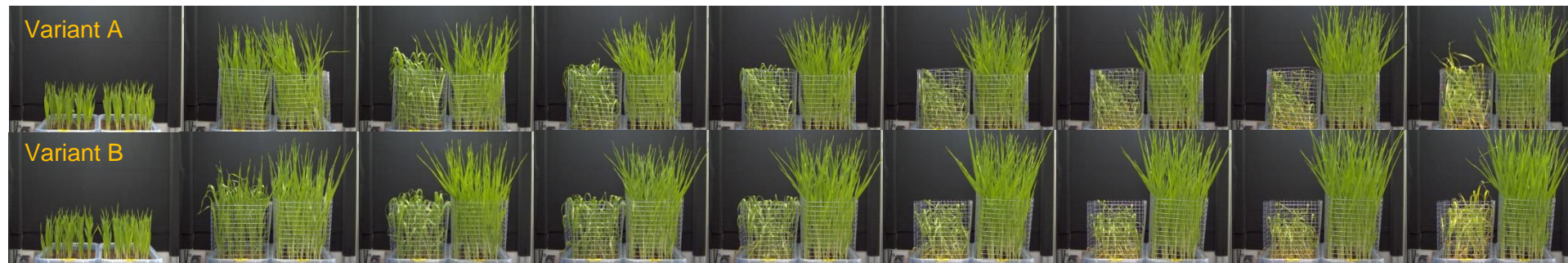




- Crop stress response
- Drought, salinity, cold
- Fast assay using RGB imaging



## Drought tolerance assay with crops



15 days

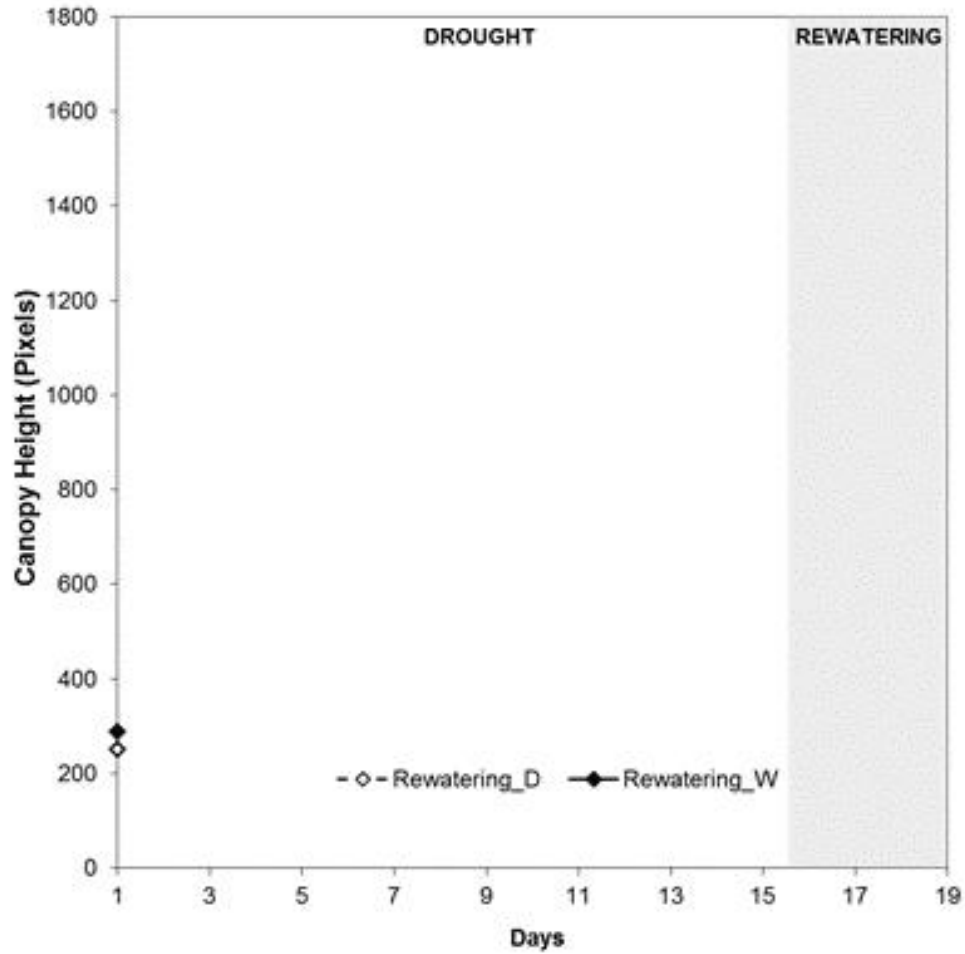
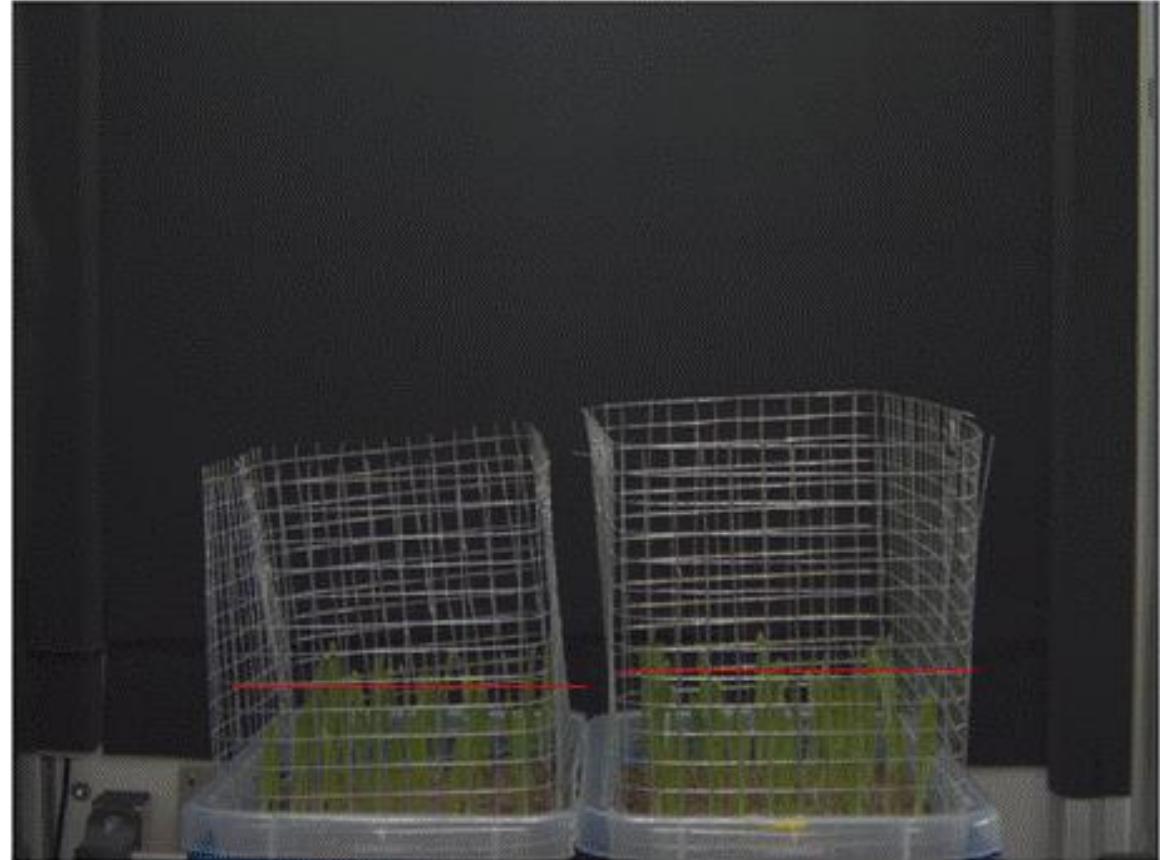
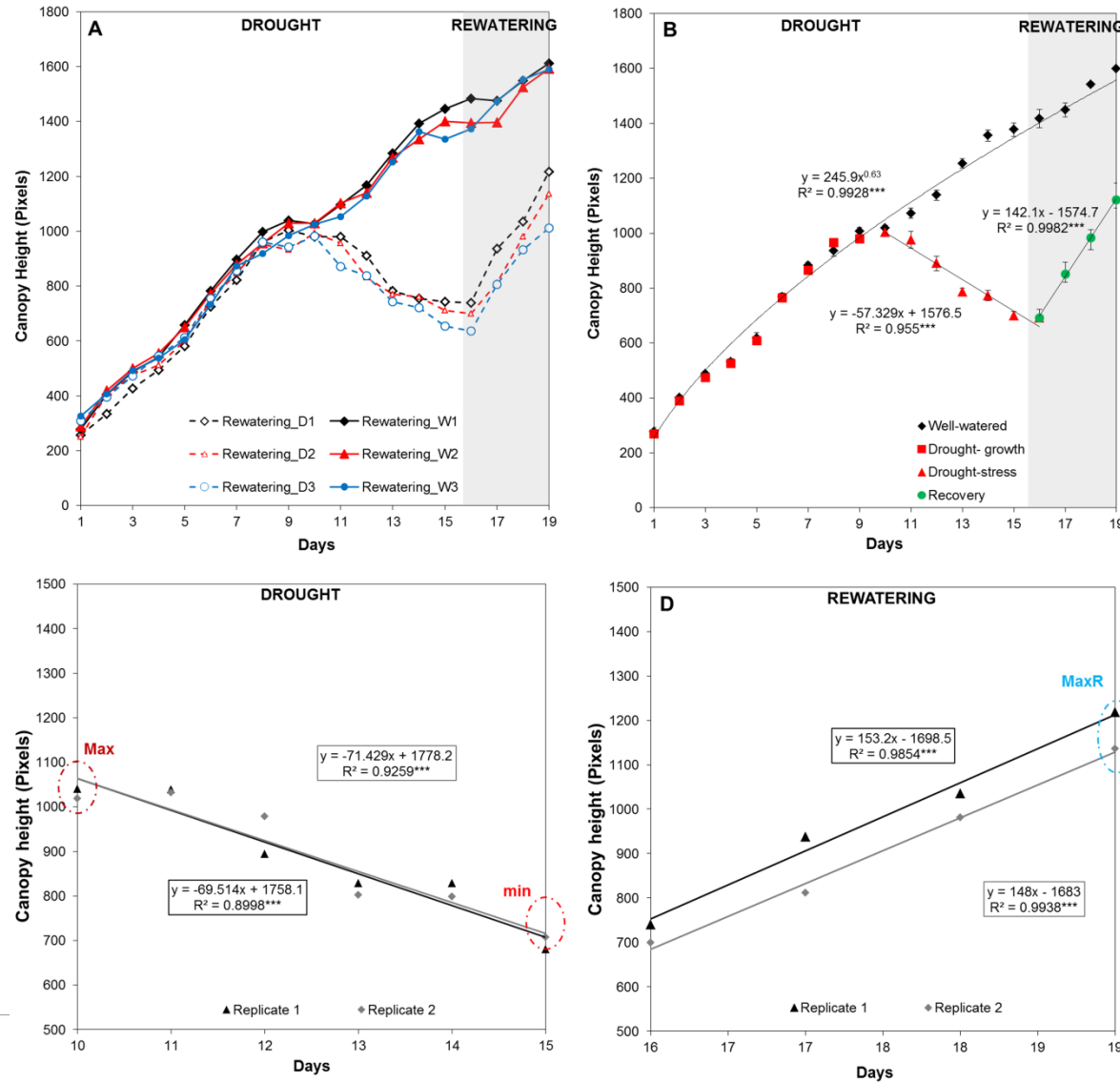
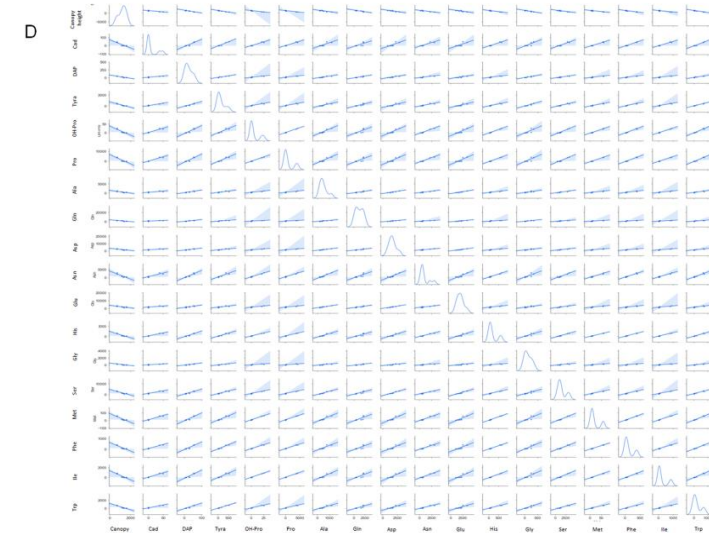
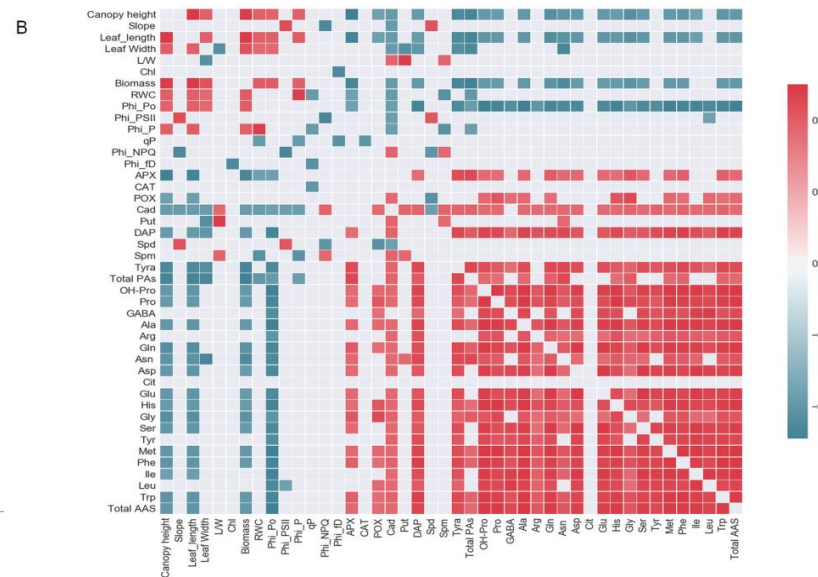
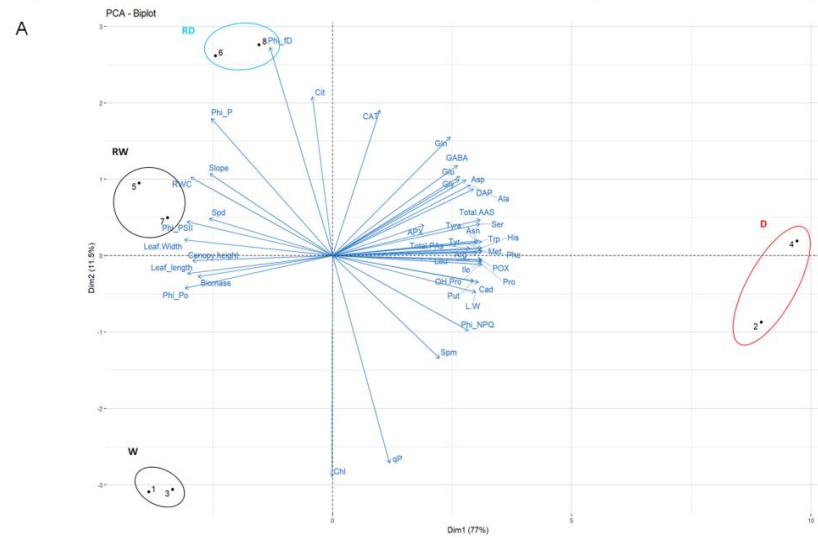


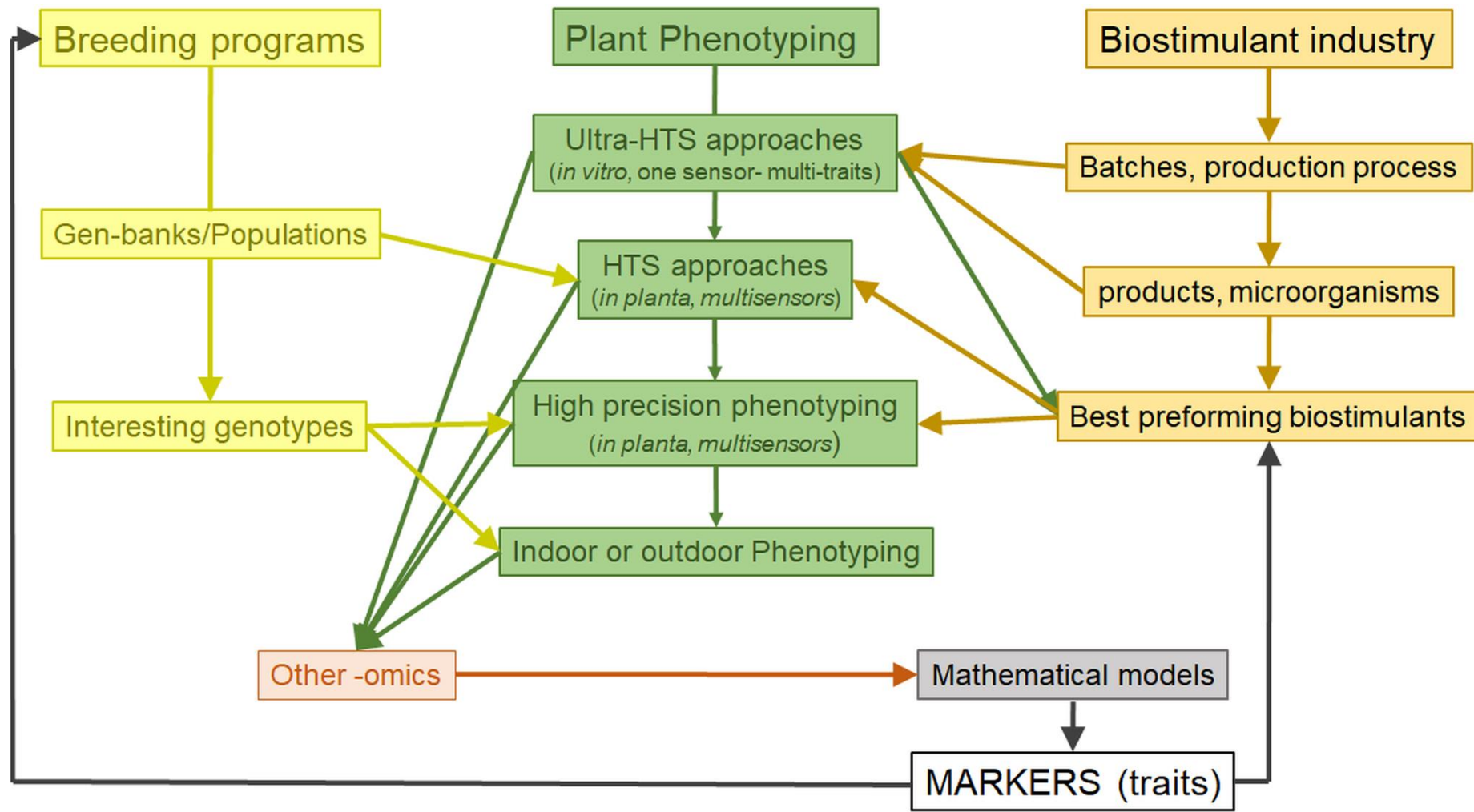
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# Suggested interconnection between “plant breeding”, “plant phenotyping” and “biostimulants”



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