Pascal Nuijten

5/4/2025

MicroRes : Microbial Induction of Plant Resilience to Drought Stress

The relevance of drought?

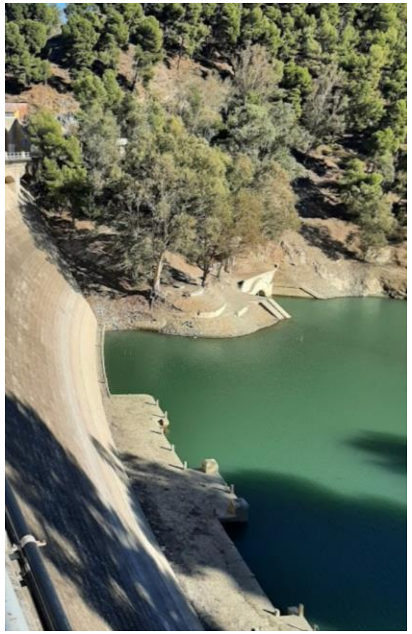
Spain: Agriculture drought losses to reach 10 billion

Nieuwsbericht | 29-08-2022 | 12:00

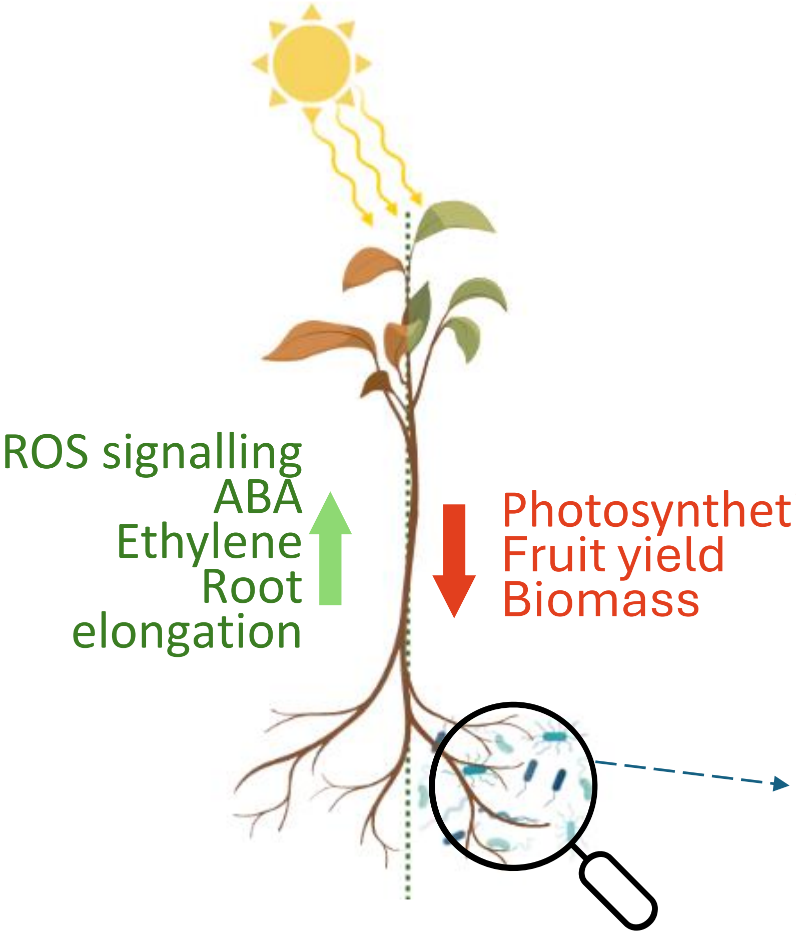
The drought in Spain is "the most severe in the last fifteen years", with 32.6% of the country in a situation of "prolonged drought", due to high temperatures and lack of rainfall. Estimates suggest that losses in the agricultural sector will be close to 10 billion euros.

What happens during drought?

2021



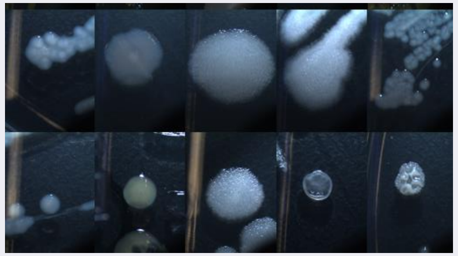
2024



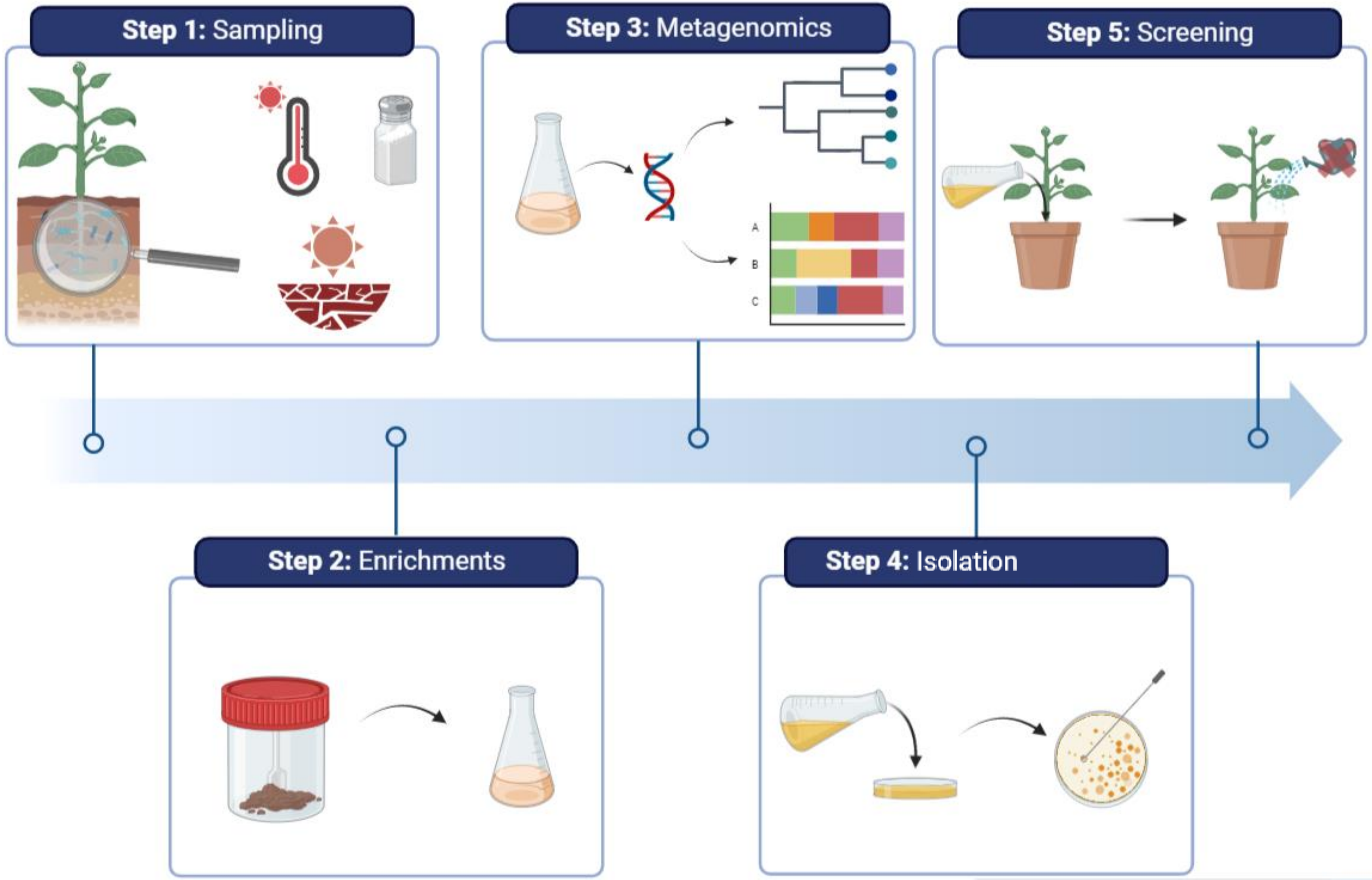
Bacterial induced
Drought **S**tress **A**lleviation (**DSA**)

- Find novel DSA bacteria
- Find novel DSA functions
- Optimize the effect
- Application in agriculture

Atacama desert

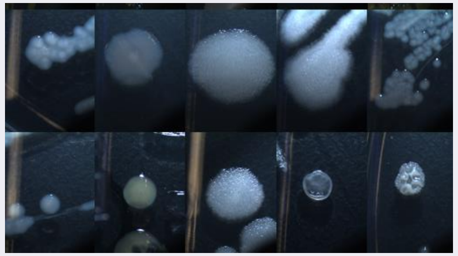


Desert extremophiles to enhance drought tolerance

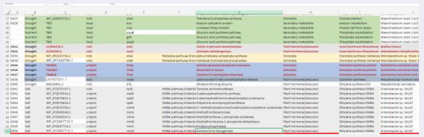


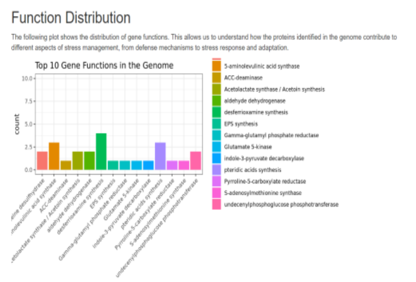
Discover novel bacteria and functions to alleviate drought stress in (crop)plants

Atacama desert



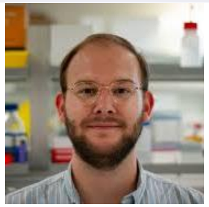
MicroStressDB





MicroRes

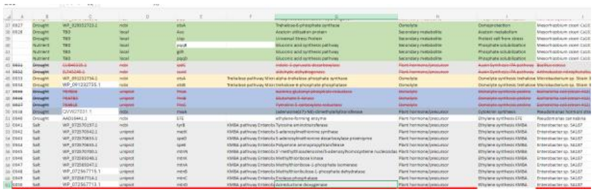
MicroStressDB: predicting abiotic stress alleviation potential



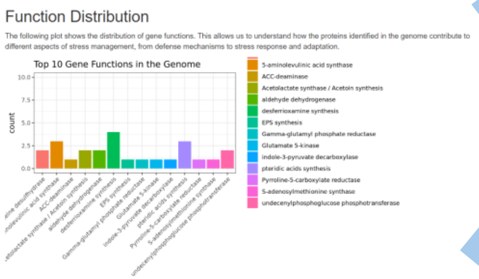
Adam Ossowicki

Guillermo Guerrero Edigo

Kevin Bretscher

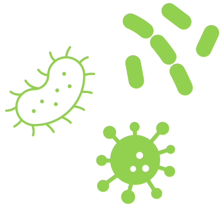


List of ~90 genes directly related to drought or salt stress

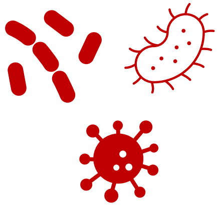


Report on functional presence and stress alleviating prediction

Stress alleviating bacteria

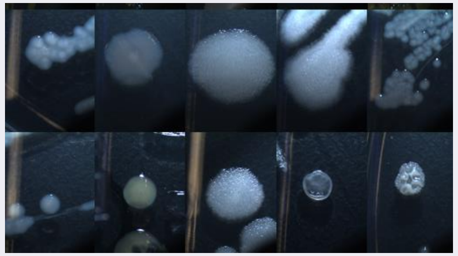


In planta validation

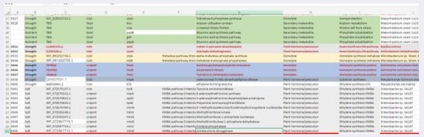


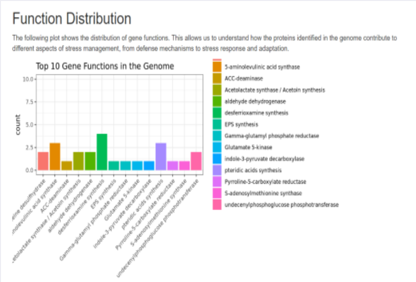
Non-stress alleviating bacteria

Atacama desert

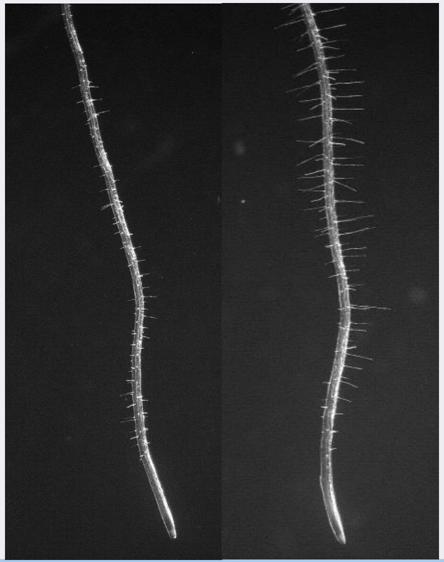


MicroStressDB

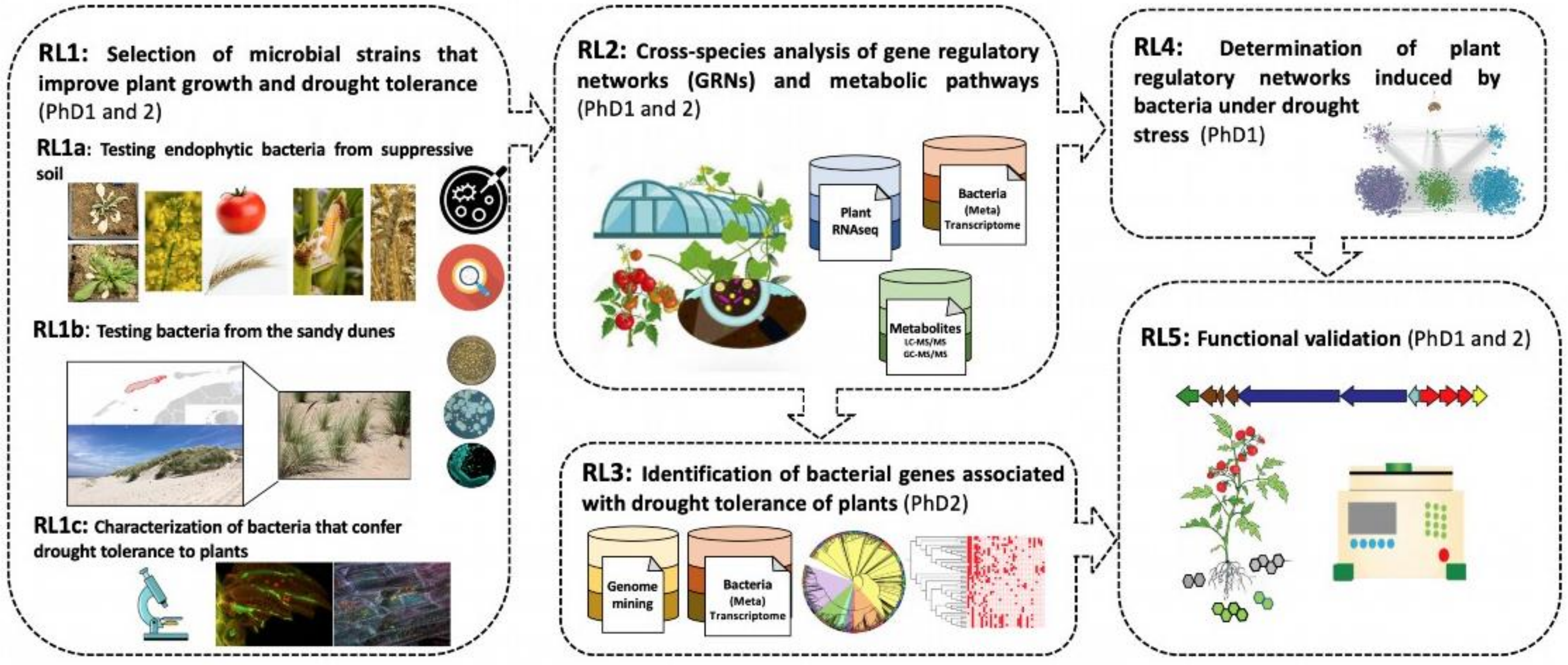




MicroRes

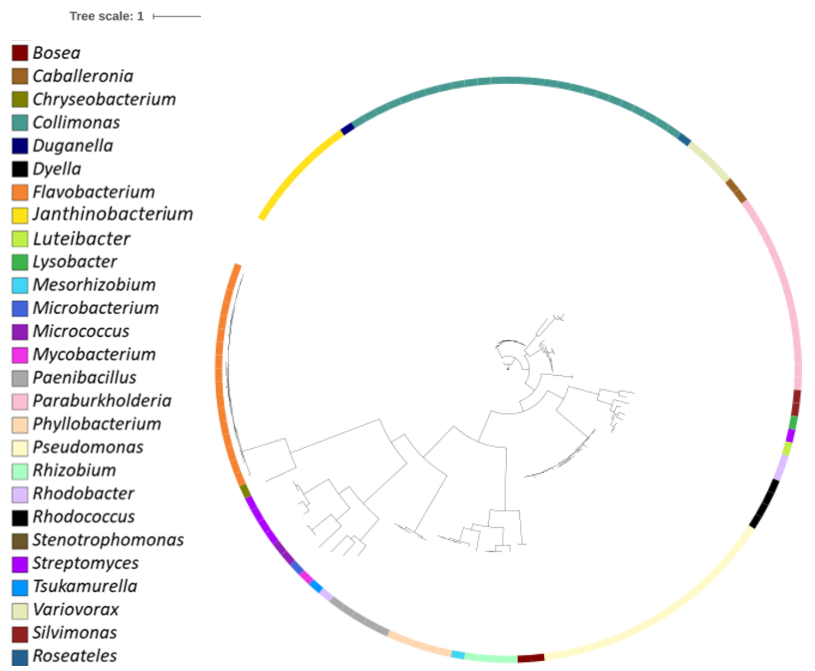


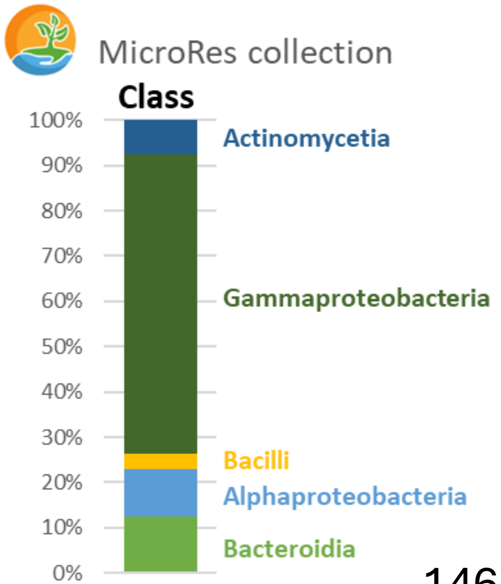
MicroRes : Microbial Induction of Plant Resilience to Drought Stress



MicroRes : Microbial Induction of Plant Resilience to Drought Stress

MicroRes Collection





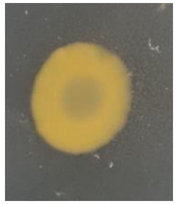
146 isolates

Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome

Science

VÍCTOR J. CARRIÓN, JUAN PEREZ-JARAMILLO, VIVIANE CORDOVEZ, VITTORIO TRACANNA, MATTIAS DE HOLLANDER, DANIEL RUIZ-BUCK, LUCAS W. MENDES, WILFRED F.J. VAN IJCKEN, RUTH GOMEZ-EXPOSITO, [...], AND JOS M. RAAIJMAKERS +8 authors Authors Info & Affiliations

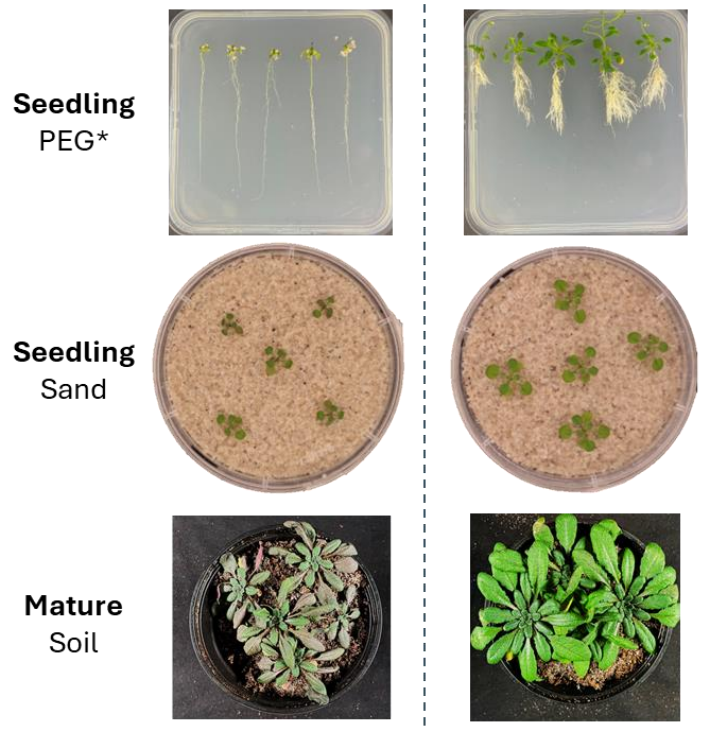
SCIENCE • 1 Nov 2019 • Vol 366, Issue 6465 • pp. 606-612 • DOI: 10.1126/science.aaw9285



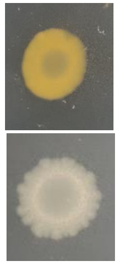
Flavobacterium sp. 98

Dr. J. Carrión | Dr. J. Raaijmakers | Dr. Balazadeh | Dr. Rahimi | Sofia Stiegert

Drought bio-assays



12 + 1 candidates

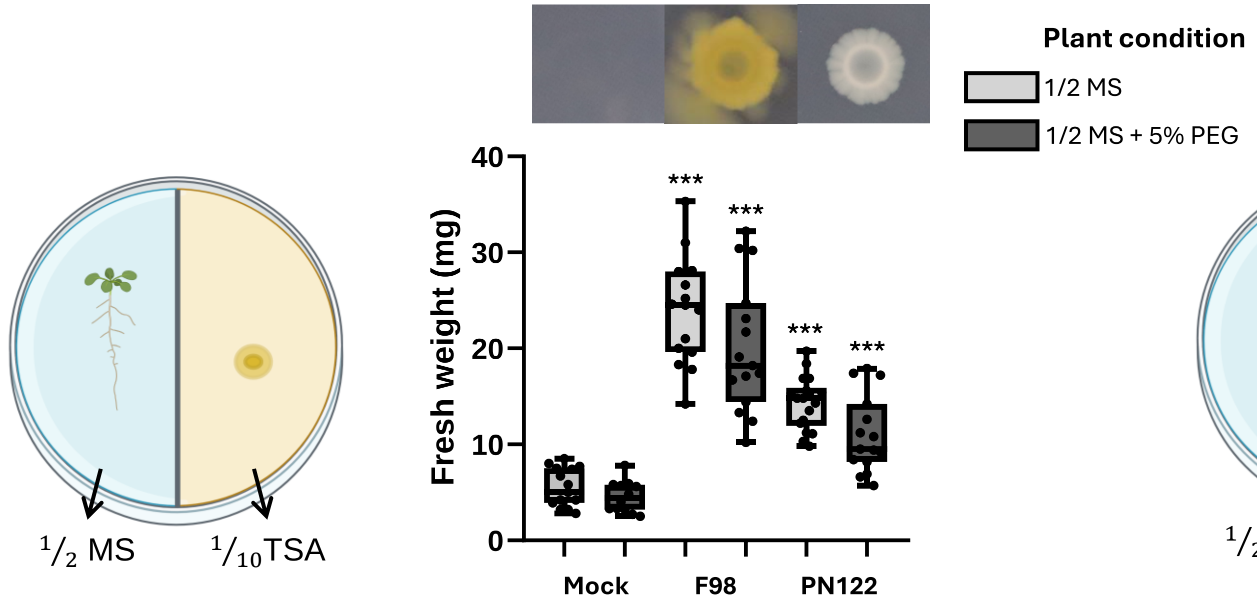


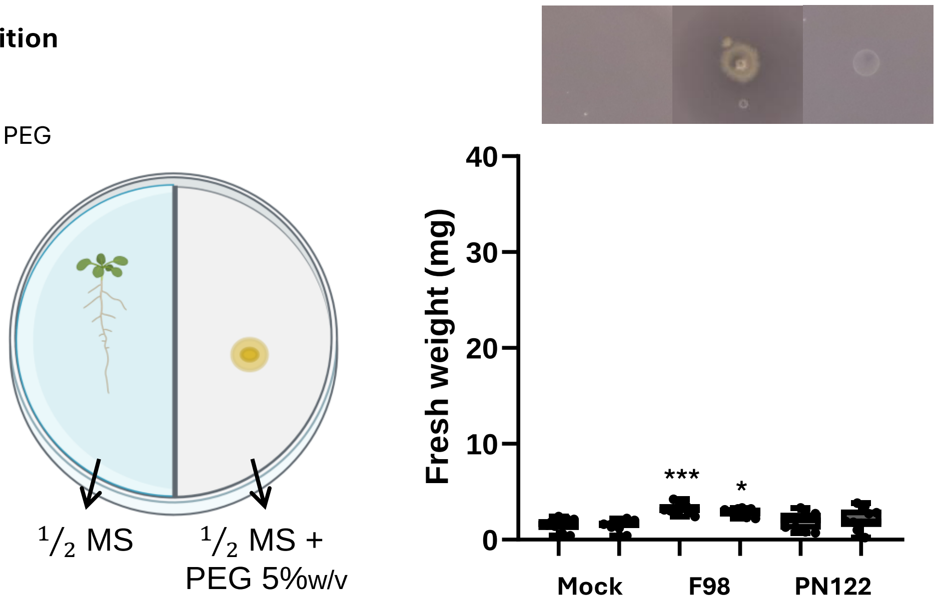
Flavobacterium sp. 98 (F98)

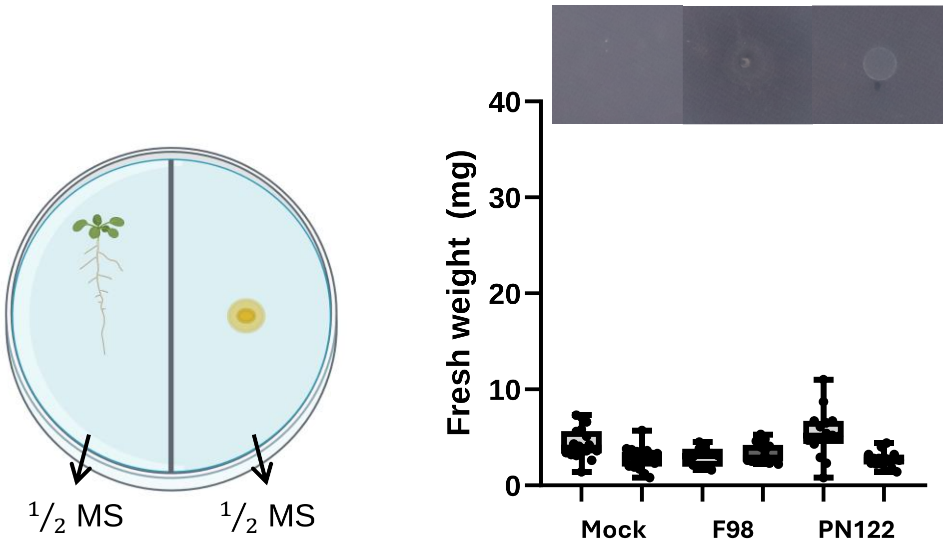
Pseudomonas sp. N122 (PN122)

*Polyethylene glycol

Potential impact of VOCs on PGP and drought stress alleviation





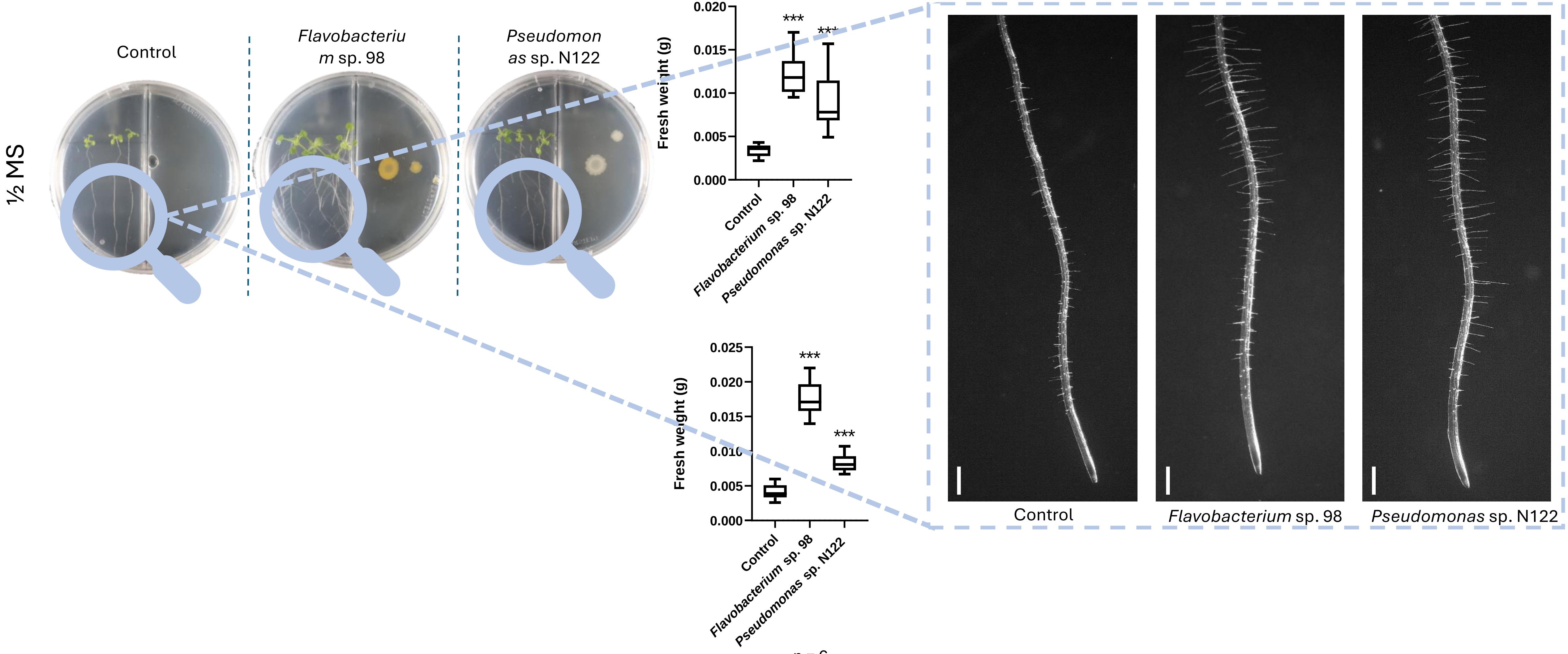


Conclusion :

F98 and PN122 show strong drought stress alleviation in rich bacterial media via VOCs

N ≥ 8, One-way ANOVA compared with relative mock treatment: *** = $p \leq 0.001$, * = $p \leq 0.05$, experiment was performed twice with similar results.

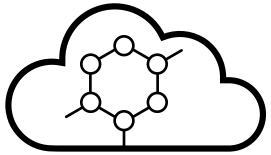
Drought stress alleviation via VOCs



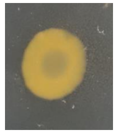
n = 6

*** = *p*-value < 0.005

Strategies to unravel the underlying VOC(s)



Pseudomonas sp. N122 (PN122)



Flavobacterium sp. 98 (F98)

Tn5-mutagenesis

Screening mutants

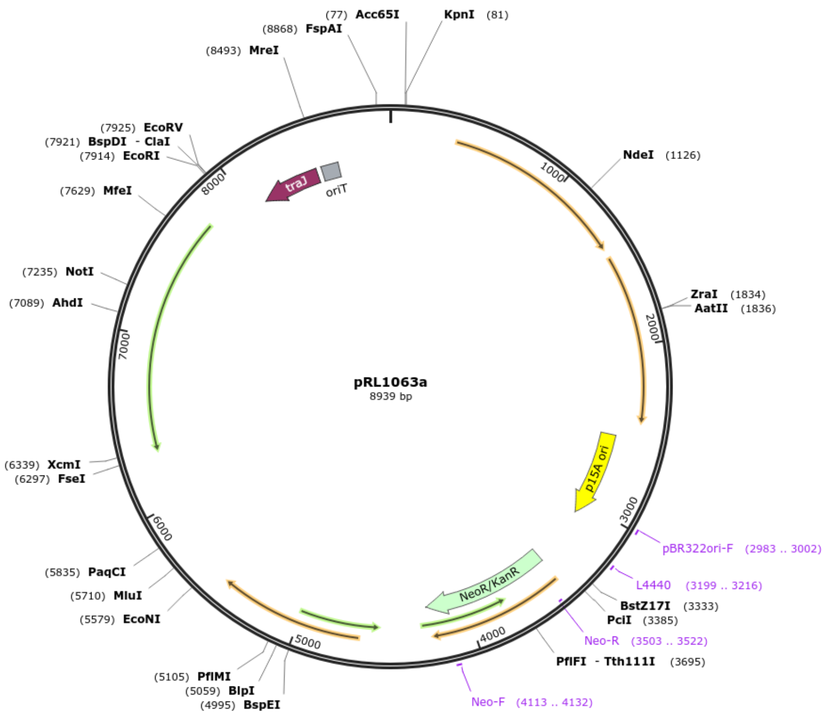
Localizing genetic markers

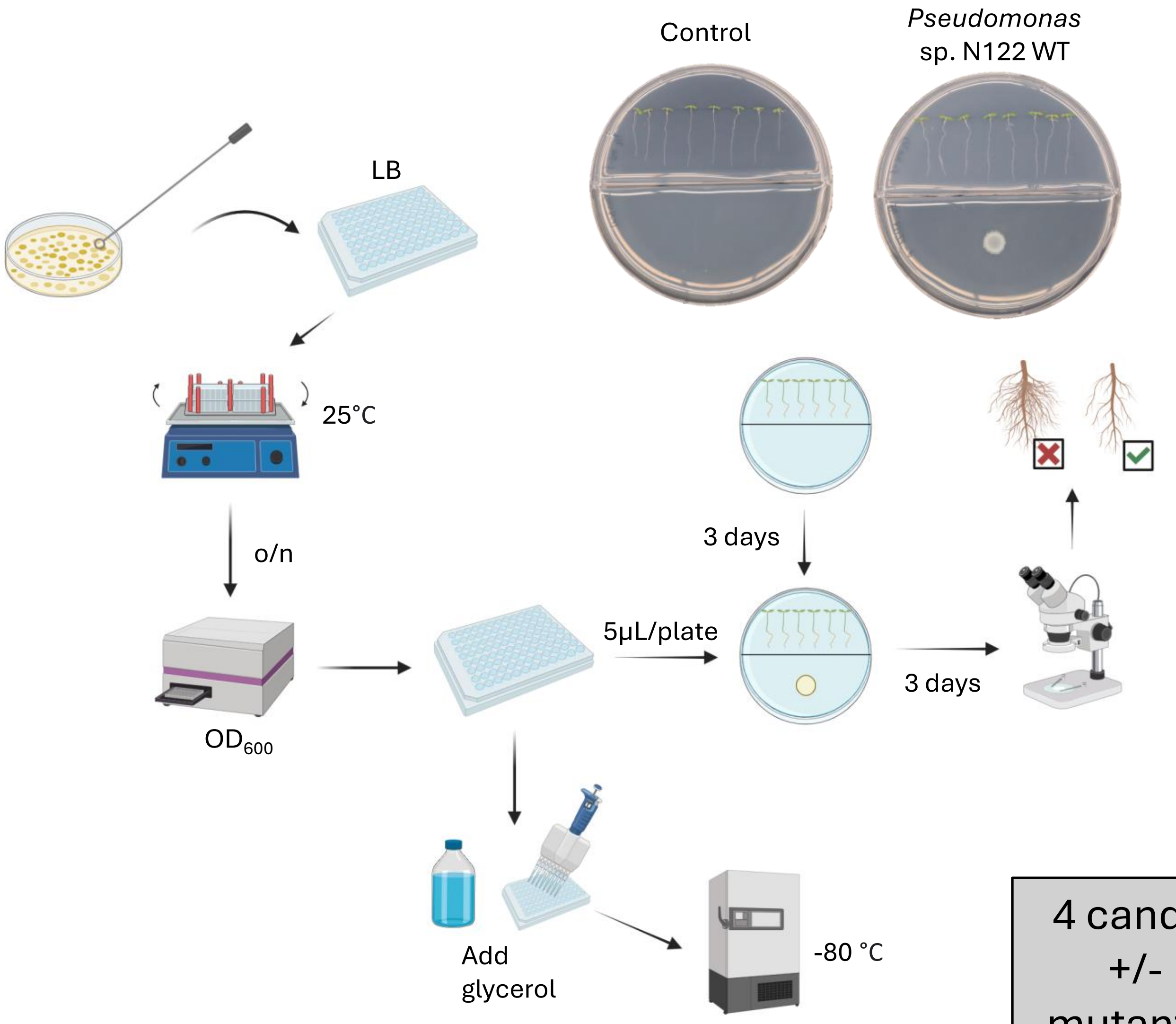
GC-MS analysis

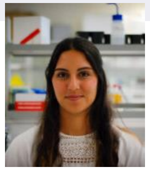
Screening VOCs

Targeted mutagenesis

Random tn5 mutagenesis to target genetic markers



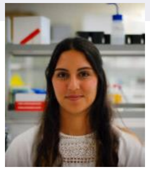


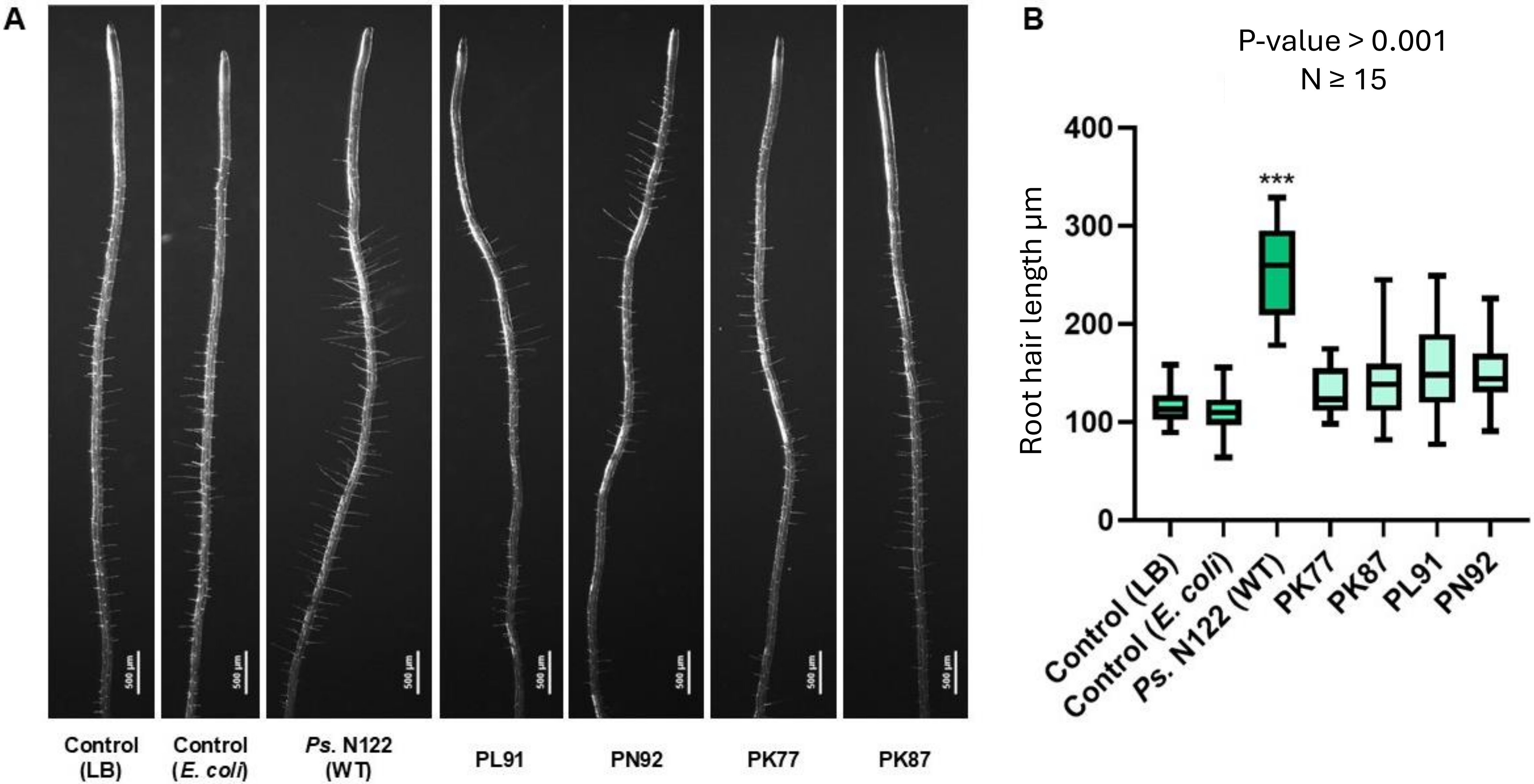


Cristina
Sarmiento

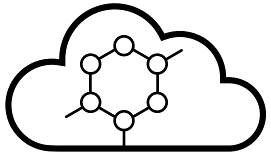
4 candidates with
+/- 1800 tn5
mutants screened

Random tn5 mutagenesis to target genetic markers

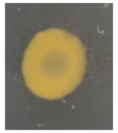

Cristina Sarmiento



Strategies to unravel the underlying VOC(s)



Pseudomonas
sp. N122 (PN122)



Flavobacterium
sp. 98 (F98)

Tn5-mutagenesis

↓

Screening mutants

↓

Localizing genetic markers

GC-MS analysis

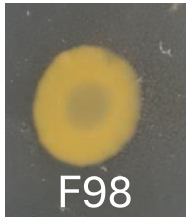
↓

Screening VOCs

↓

Targeted mutagenesis

Mining the headspace of F98 and PN122 for VOCs

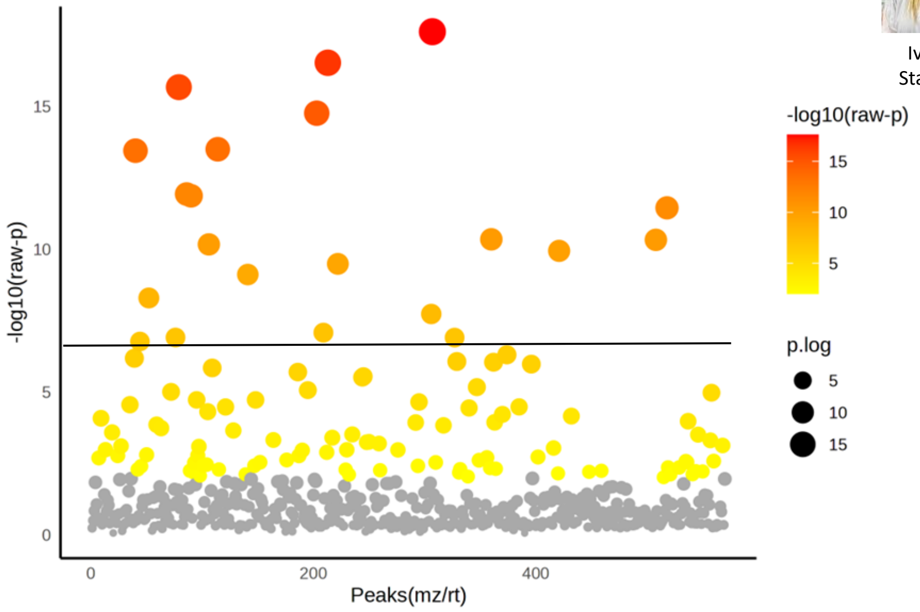


NIOO

Agilent Technologies
MassHunter Workstation

MZmine 3

MetaboAnalyst 6.0



25 Features*

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

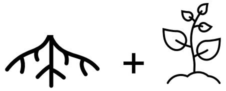
22 VOCs

Ivana Staiano

Dr. Christina Papazlatani

Muhammad Syamsu Rizaludin

Xinya Pan



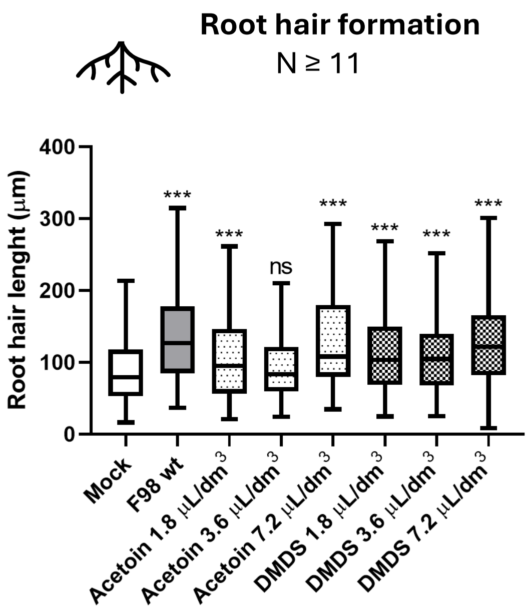
Root hair formation

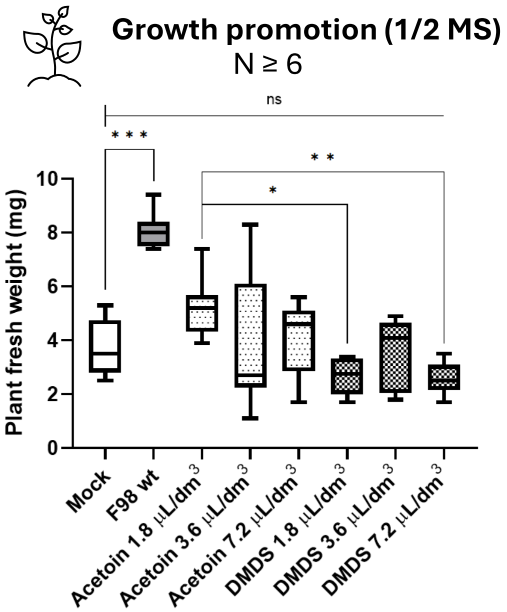
+ PGP/ Drought stress alleviation

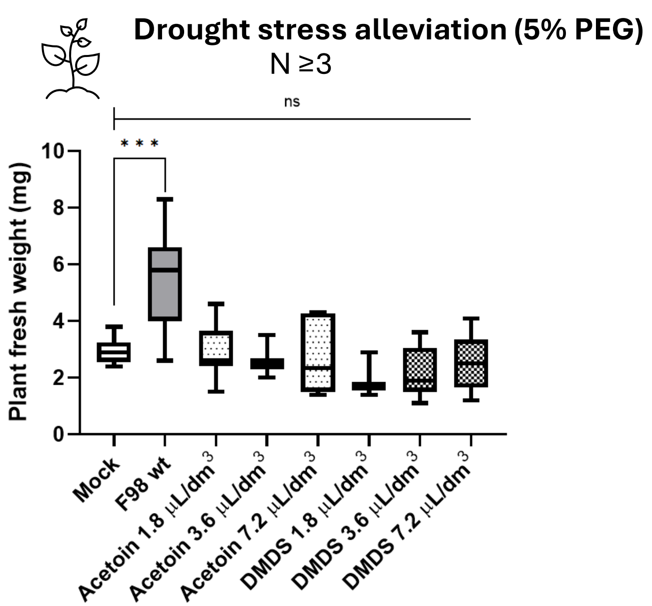
- 1-Decene
- 1-Undecene
- Methyl Thiocyanate
- 3-Mehyl-1-butanol
- 3- Pentanone
- 3-Hydroxy-2-butanone
- Methyl Isovalerate
- S-Methyl methanesulfonothioate
- 5-Butanoic acid
- Isoamyl Acetate
- 3-Methyl-2-butanone
- Dimethyl Trisulfide
- 2,4 Dithiapentane
- **Dimethyl Disulfide (DMDS)**
- **2-butanone, 3 hydroxy (Acetoin)**

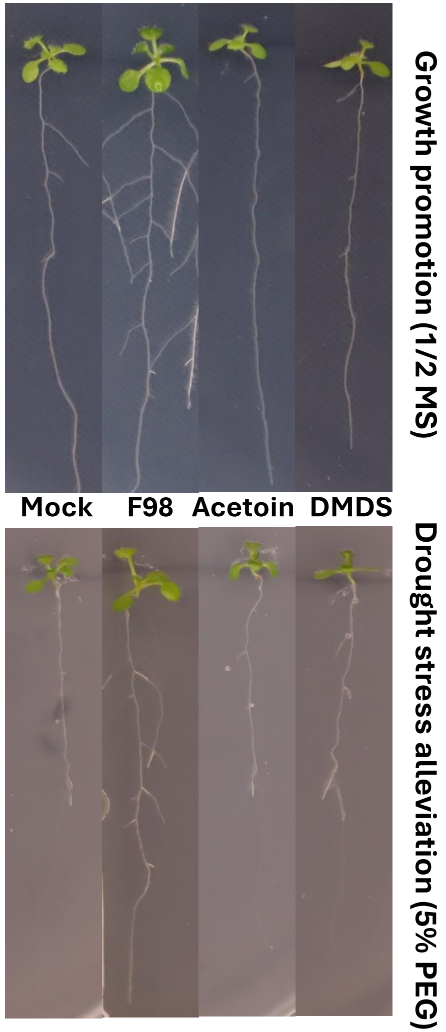
*P-value ≥ 0.05

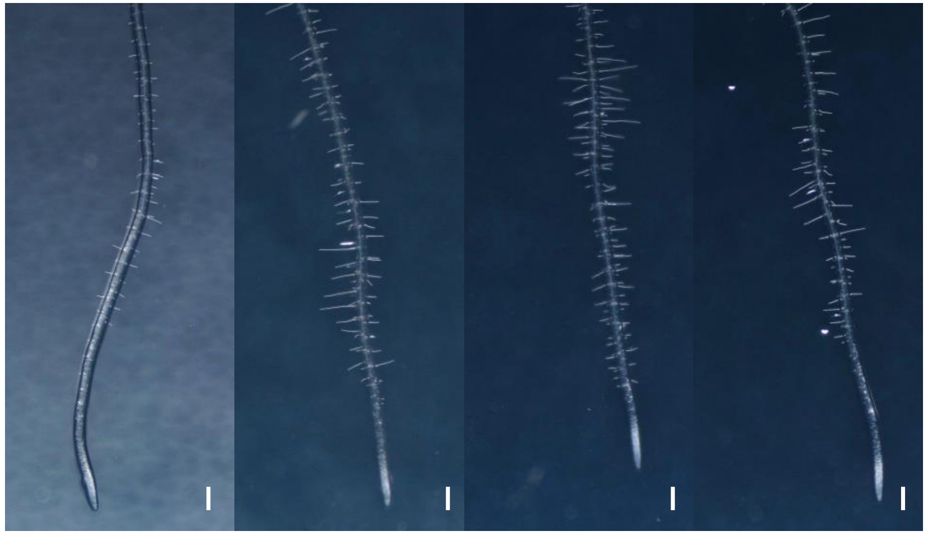
The effect of Acetoin and DMDS on *A. thaliana*











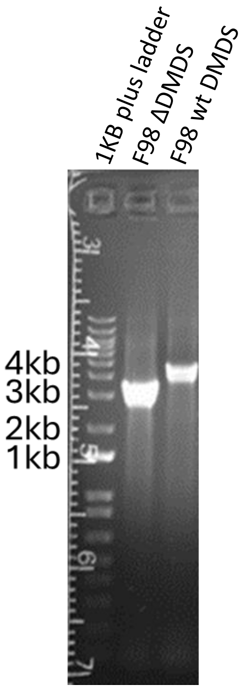
Mock F98 Acetoin DMDS

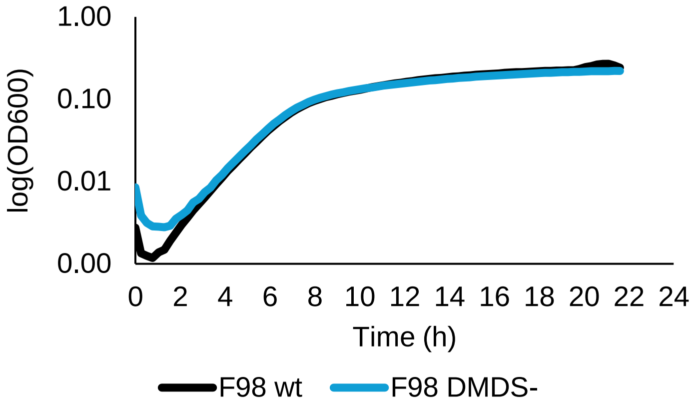
Conclusion :

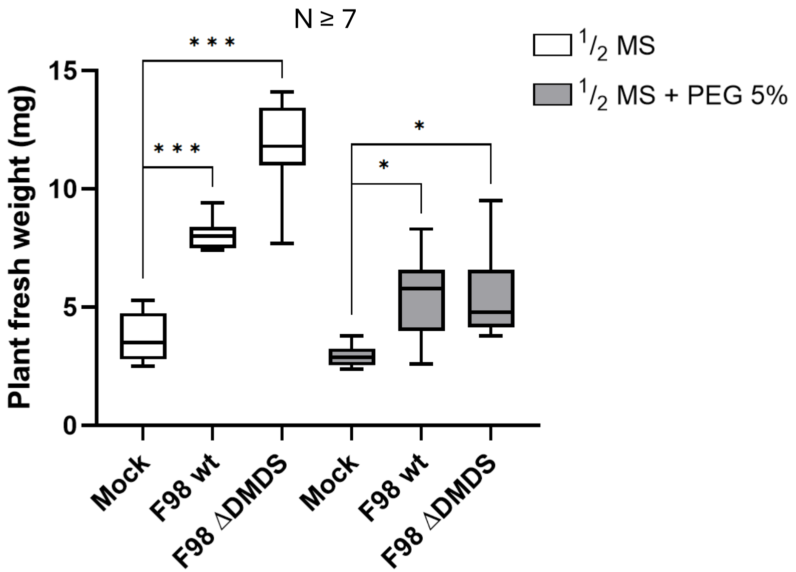
Acetoin and DMDS <u>mimic root hair formation</u> but <u>not growth promotion/drought stress alleviation</u>

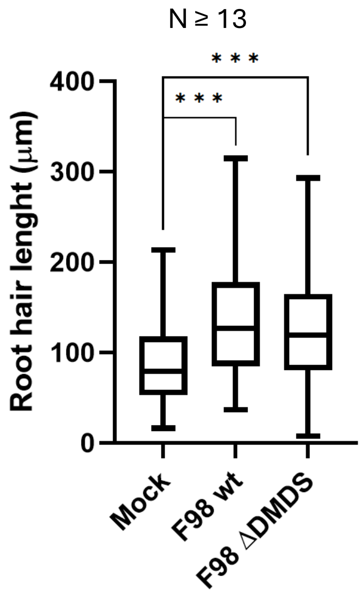
One-way ANOVA compared with relative mock treatment: *** = p ≤ 0.001, ** = p ≤ 0.01 , * = p ≤ 0.05

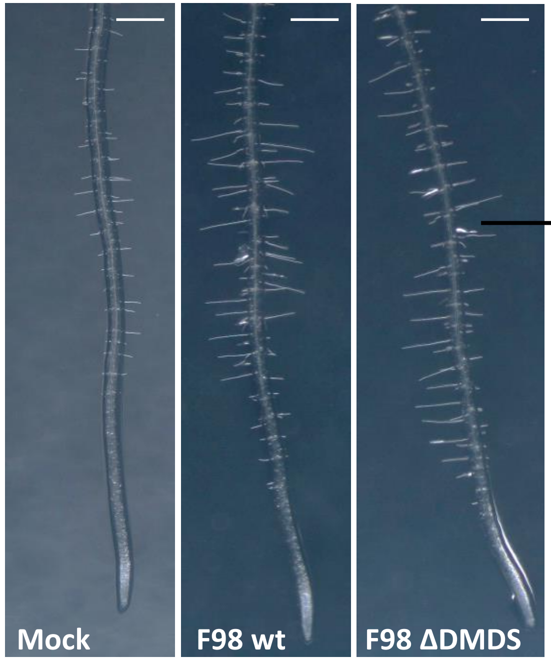
Targeted knock-out mutants in Acetoin and DMDS biosynthesis











Conclusion :
Mutants in L-methionine-γ-lyase gene involved in DMDS biosynthesis still confer drought stress alleviation and root hair formation *in vitro*

Is F98 ΔDMDS still producing DMDS?

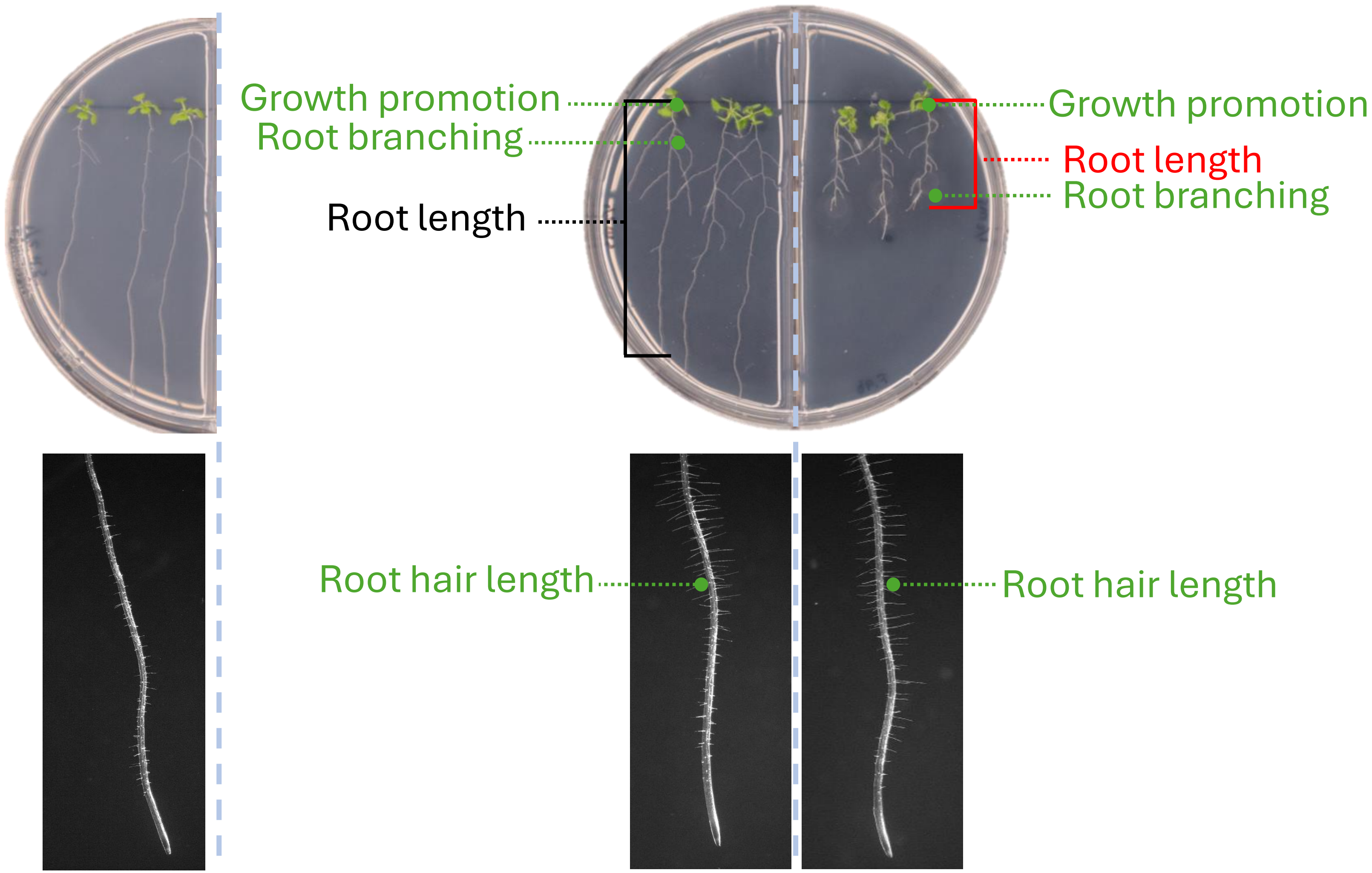
NIOO

One-way ANOVA compared with relative mock treatment: *** = p ≤ 0.001, ** = p ≤ 0.01 , * = p ≤ 0.05

MicroRes : Microbial Induction of Plant Resilience to Drought Stress

Ivana Staiano

Control ($MgSO_4$) | **Volatile (F98)** | **Diffusible (F98)**



*Plates are ½ MS

Conclusion

F98 is shaping the root of *A. thaliana* in two different ways :

Volatile
+ Growth promotion
+ Root branching
+ Root hair formation

Diffusible (contact)
- Root length

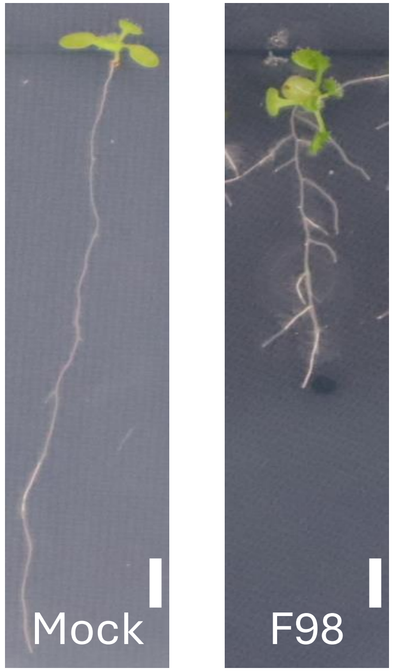
So we are dealing with multiple mechanisms!

Approaches to target candidate genes involved in our phenotype

Volatile

- ❑ Root branching
- ❑ Root hair formation*

Direct contact

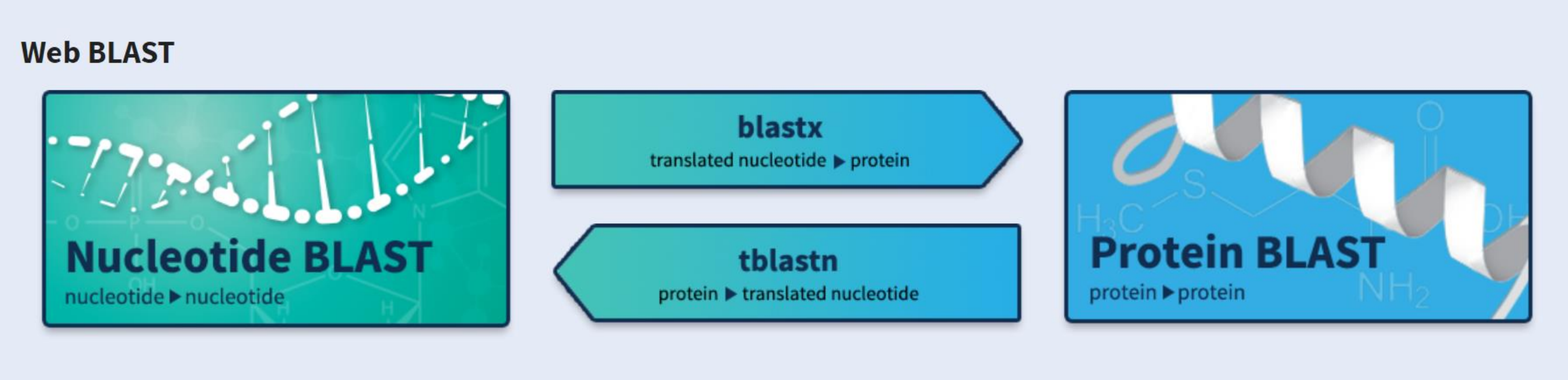


- ❑ Additional Root branching?
- ❑ Shorter primary root

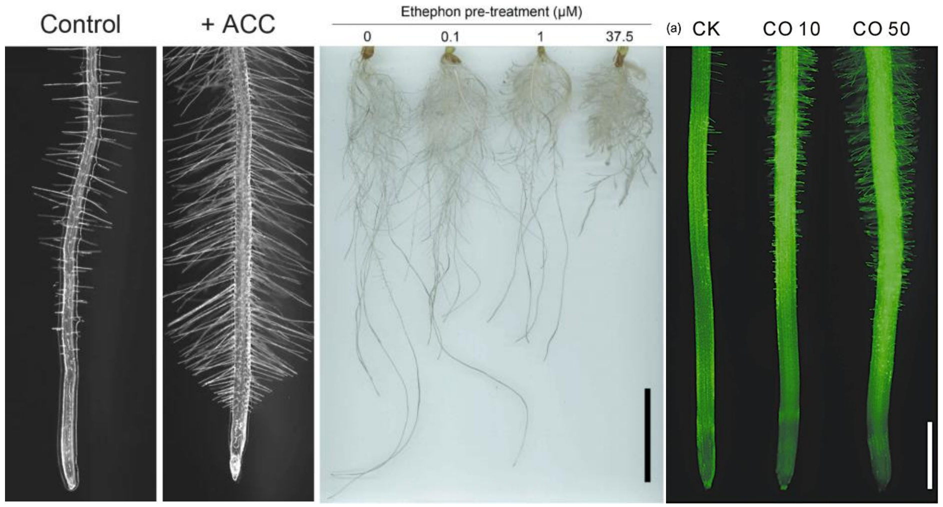
?

BLAST

Literature research BLAST analysis in



- Ethylene
- Acetylene
- 1-Aminocyclopropane-1-carboxylate (ACC)
- Ethephon
- Triplin
- Propylene
- carbon monoxide
- Isocyanides
- silver nitrate
- 1-methylcyclopropene (1-MCP)
- Jasmonic acid
- methyl jasmonate



ACC Ethephon Carbon monoxide

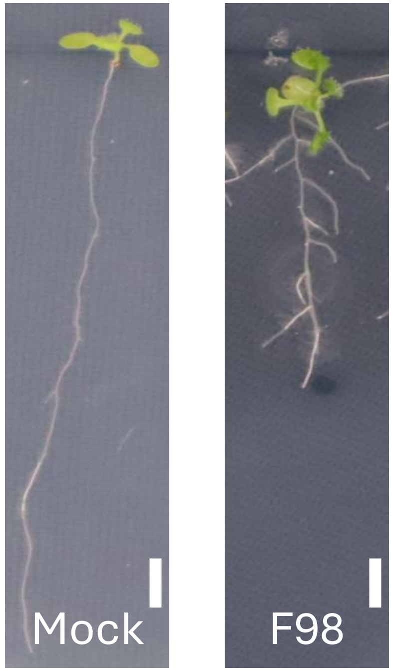
Many target genes acquired

Approaches to target candidate genes involved in our phenotype

Volatile

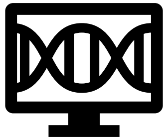
- ❑ Root branching
- ❑ Root hair formation*

Direct contact



- ❑ Additional Root branching?
- ❑ Shorter primary root

?



BLAST

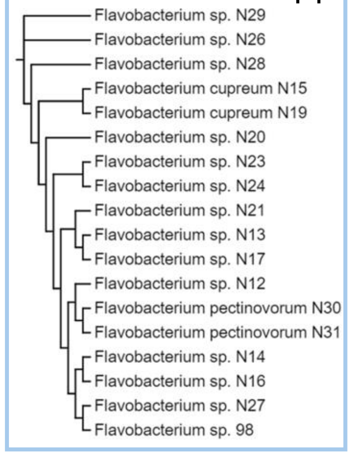
BacLIFE

BacLIFE identify the genes involved in drought stress alleviation

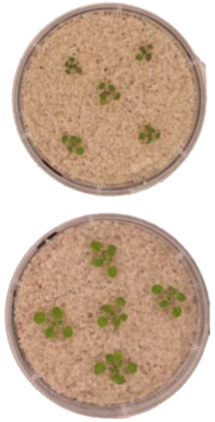
Guillermo Guerrero Edigo

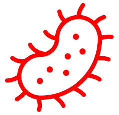
146 bacterial isolates

16x *Flavobacterium* spp.

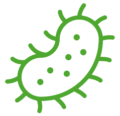


Sand Drought bio-assay



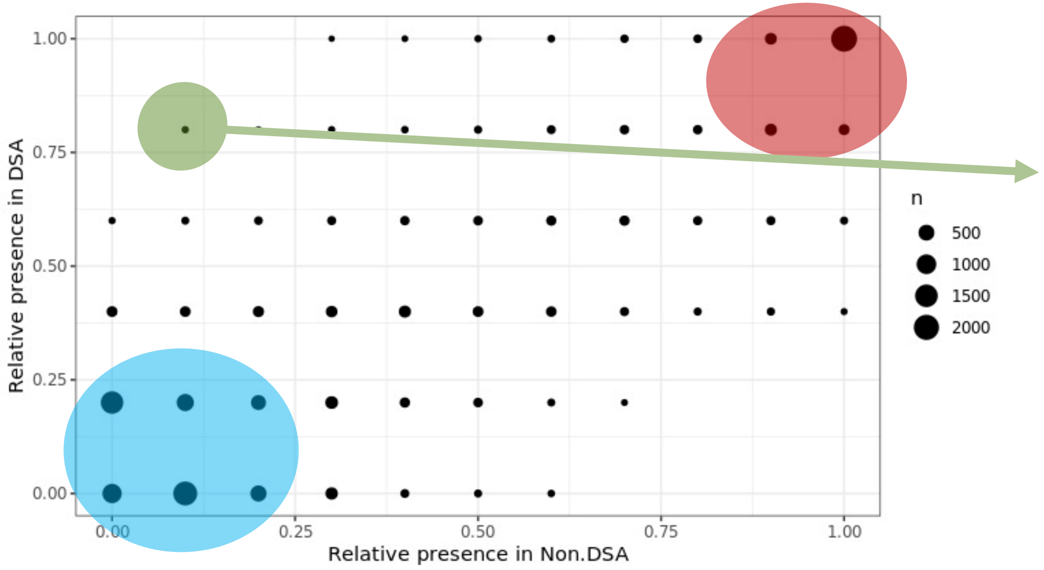


11x *Flavobacterium* spp.



5x *Flavobacterium* spp.

Comparative genomics: BacLife



6 target genes acquired

Core genome

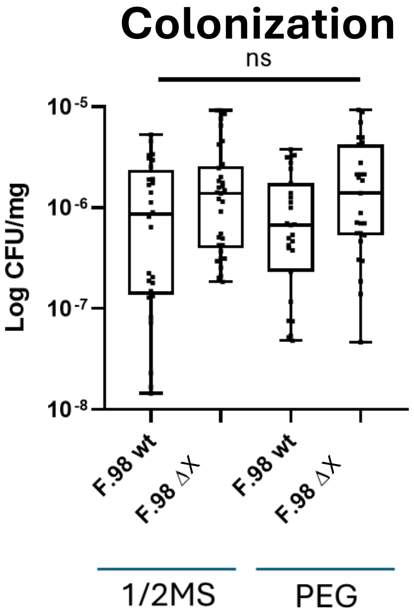
Strain-specific genes

Candidate genes

DSA = **D**rought **S**tress **A**lleviation

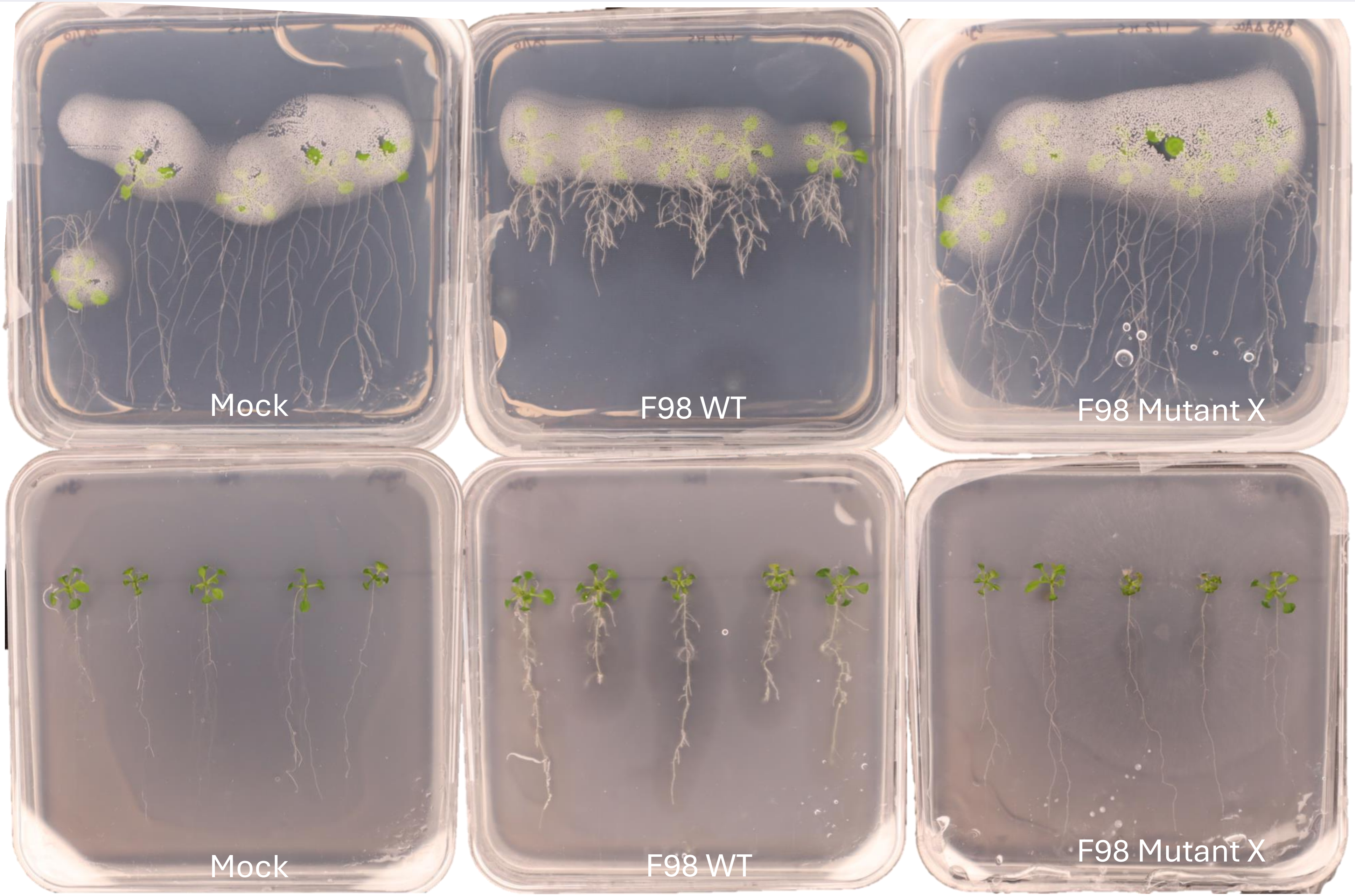
Mutant X was not able to shape the root as F98 WT

Ivana Staiano



½ MS

PEG

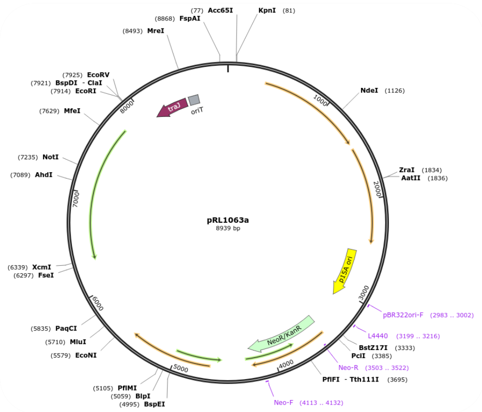



*Front view
Mock ($MgSO_4$)
F98 WT
F98 ΔX
Experiments are still on going!
*Top view

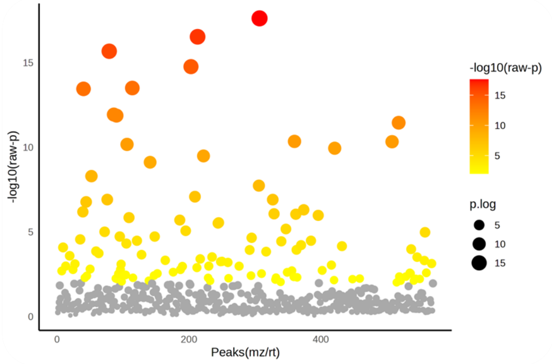
Future steps

Aim : Understanding the mechanisms of PN122 and F98 to alleviate drought stress by root architecture alterations

- Localize TN5 transposon in PN92



- Mimic the volatome of F98 WT to target key volatile(s)



- Decipher key differences of F98 WT and F98 mutant X

Thank you!

Prof.dr. J.M. Raaijmakers

Dr. S. Balazadeh

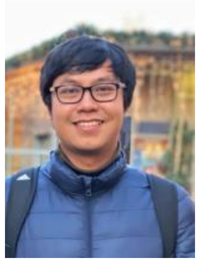
Dr. J. A. Gutiérrez Barranquero

Dr A. Rahimi

Dr. Christina Papazlatani

Dr J. Hierrezuelo León

Sofia Stiegert



Muhammad Syamsu Rizaludin

Universiteit Leiden

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