



Pascal Nuijten

5/4/2025



Universiteit
Leiden



MicroRes : Microbial Induction of Plant Resilience to Drought Stress

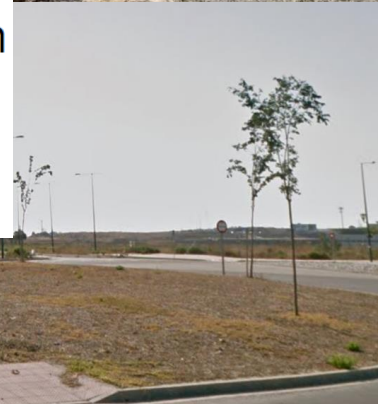
The relevance of drought?



Spain: Agriculture drought losses to reach 10 billion

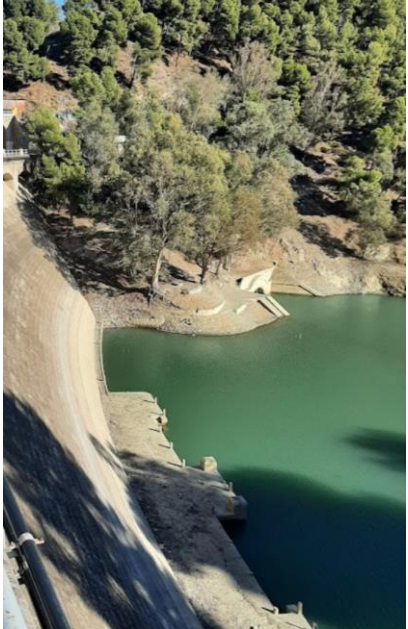
Nieuwsbericht | 29-08-2022 | 12:00

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.

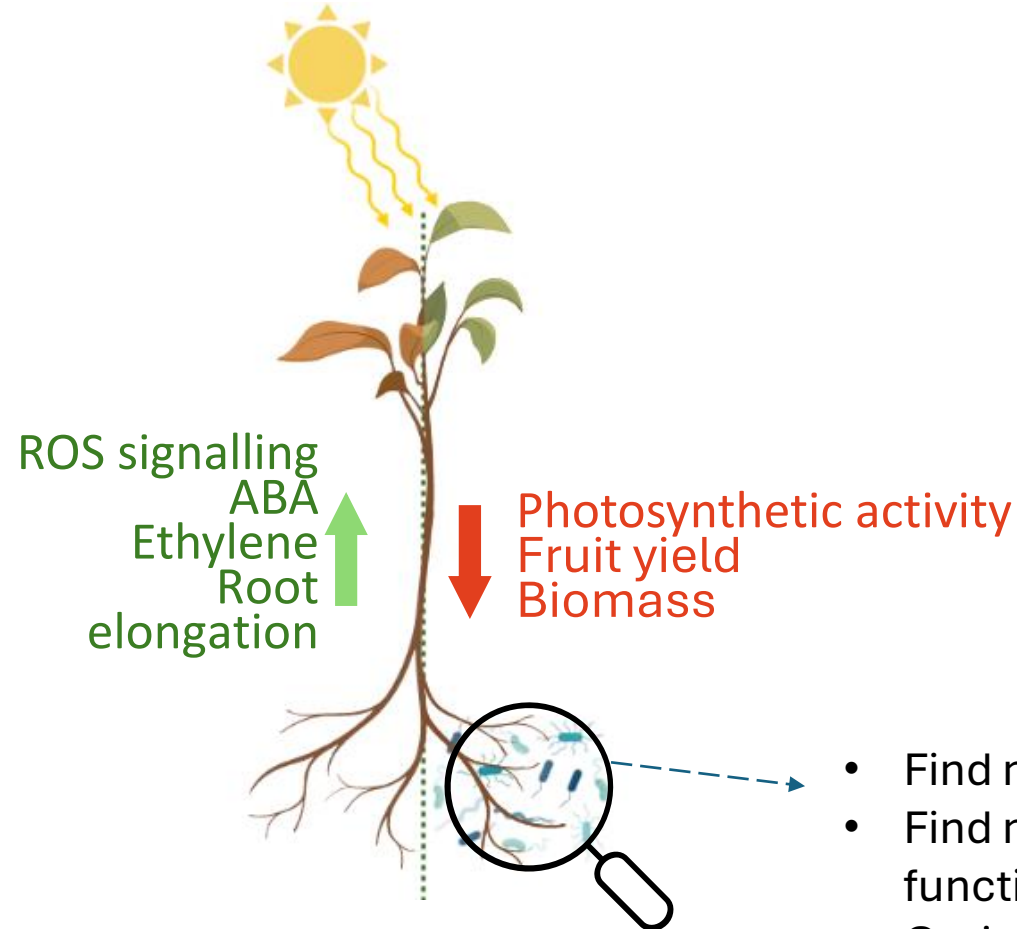


What happens during drought?

2021



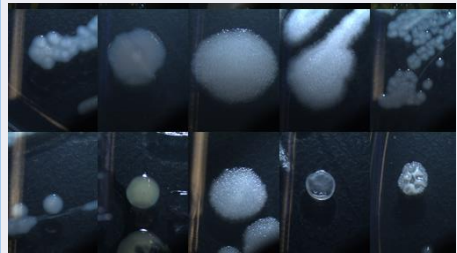
2024



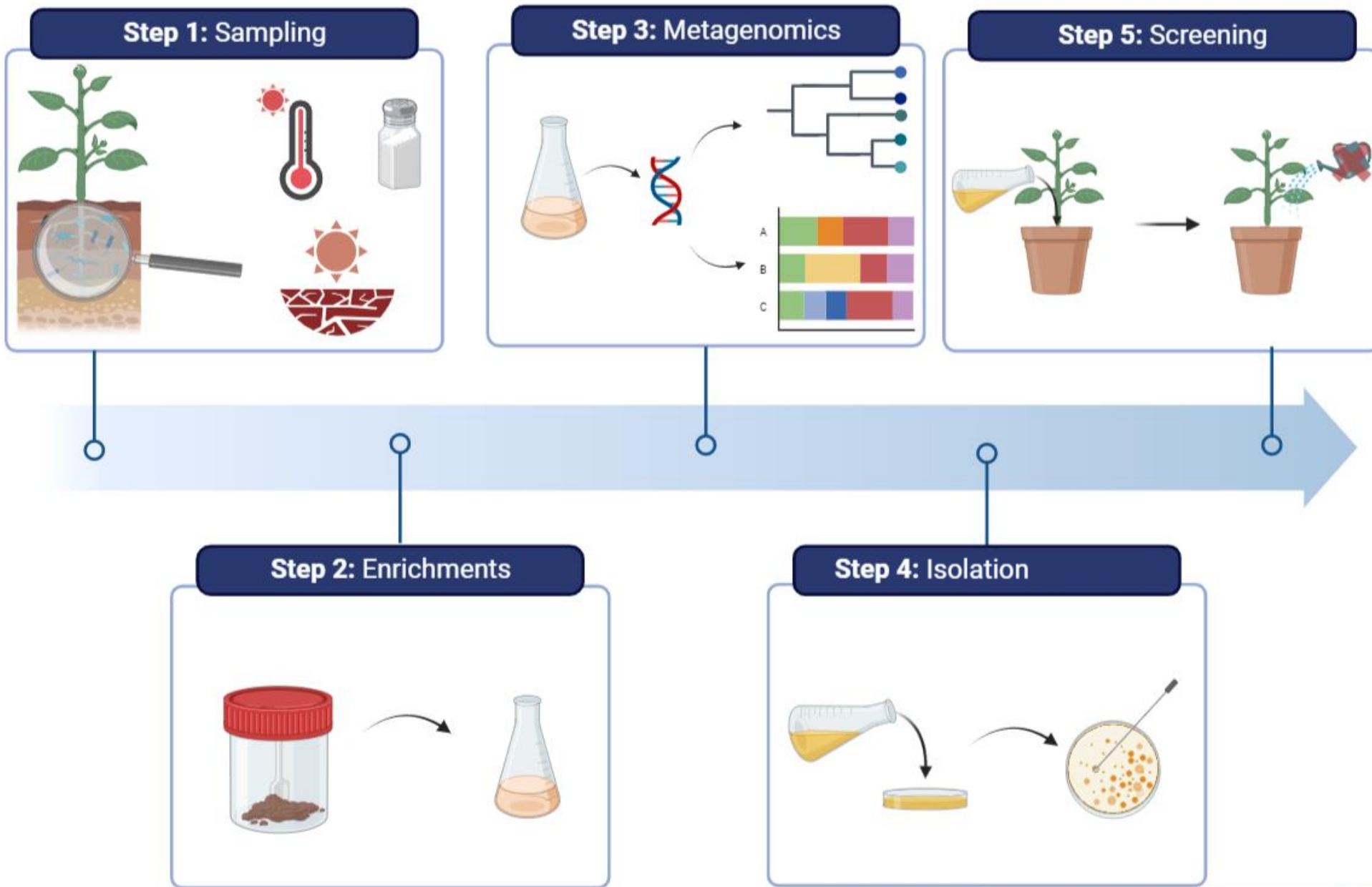
Bacterial induced
Drought Stress Alleviation (DSA)

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

Atacama desert

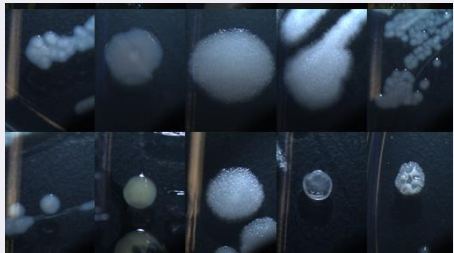


Desert extremophiles to enhance drought tolerance



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

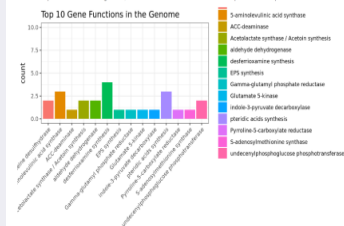




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The following plot shows the distribution of gene functions. This allows us to understand how the proteins identified in the genome contribute to different aspects of stress management, from defense mechanisms to stress response and adaptation.



MicroRes

MicroStressDB: predicting abiotic stress alleviation potential



Adam
Ossowicki



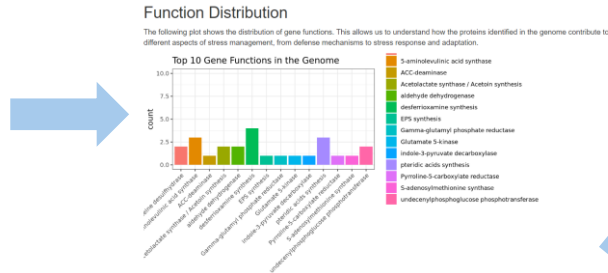
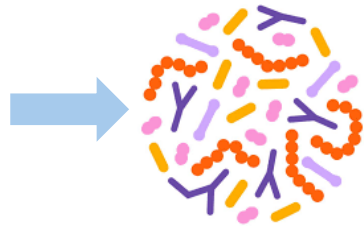
Guillermo
Guerrero
Edigo



Kevin
Bretscher

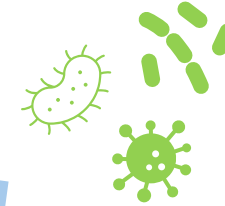
Gene ID	Gene Name	Gene Description	Gene Type	Gene Status	Gene Function	Gene Location	Gene Size	Gene GC Content	Gene GC Skew	Gene GC Bias	Gene GC Bias2	Gene GC Bias3	Gene GC Bias4	Gene GC Bias5	Gene GC Bias6	Gene GC Bias7	Gene GC Bias8	Gene GC Bias9	Gene GC Bias10	Gene GC Bias11	Gene GC Bias12	Gene GC Bias13	Gene GC Bias14	Gene GC Bias15	Gene GC Bias16	Gene GC Bias17	Gene GC Bias18	Gene GC Bias19	Gene GC Bias20
AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010	AT1G01010

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

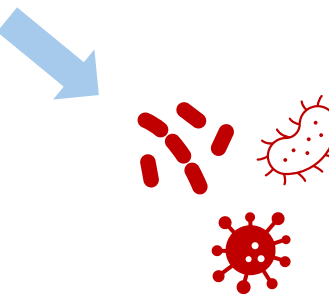


Report on functional presence and
stress alleviating prediction

Stress alleviating bacteria

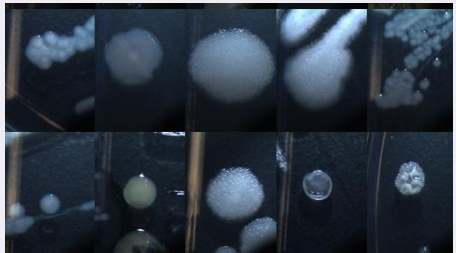


In planta validation

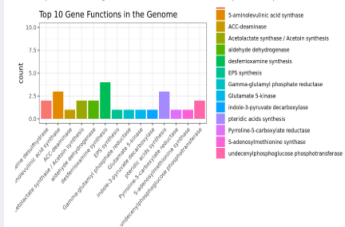


Non-stress alleviating bacteria



[illegible]

The following plot shows the distribution of gene functions. This allows us to understand how the proteins identified in the genome contribute to different aspects of stress management, from defense mechanisms to stress response and adaptation.



MicroRes : Microbial Induction of Plant Resilience to Drought Stress

RL1: Selection of microbial strains that improve plant growth and drought tolerance (PhD1 and 2)

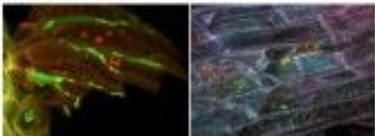
RL1a: Testing endophytic bacteria from suppressive soil



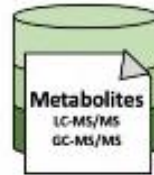
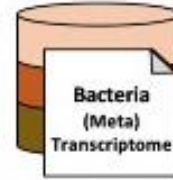
RL1b: Testing bacteria from the sandy dunes



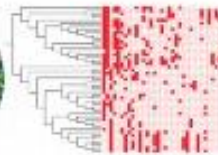
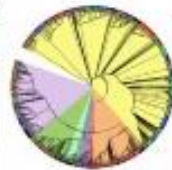
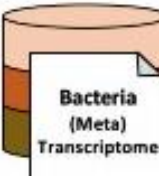
RL1c: Characterization of bacteria that confer drought tolerance to plants



RL2: Cross-species analysis of gene regulatory networks (GRNs) and metabolic pathways (PhD1 and 2)



RL3: Identification of bacterial genes associated with drought tolerance of plants (PhD2)



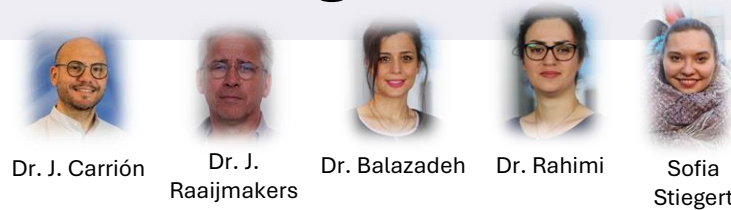
RL4: Determination of plant regulatory networks induced by bacteria under drought stress (PhD1)



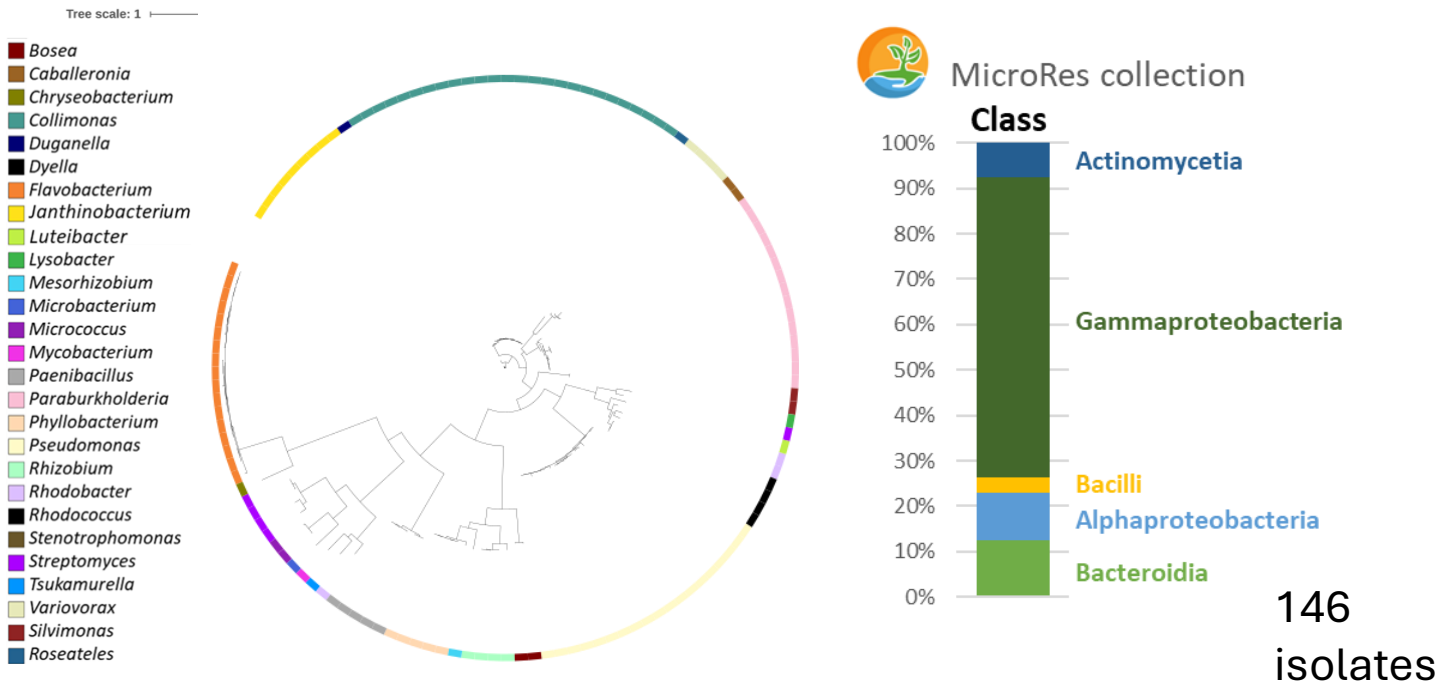
RL5: Functional validation (PhD1 and 2)



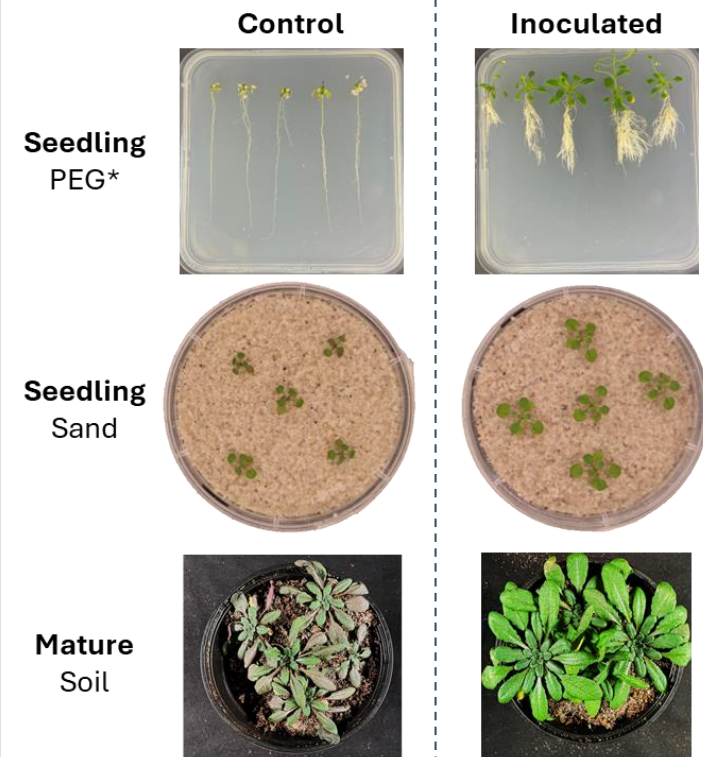
MicroRes : Microbial Induction of Plant Resilience to Drought Stress



MicroRes Collection



Drought bio-assays



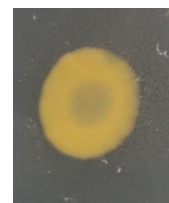
Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome

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](#)

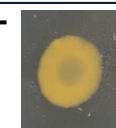
SCIENCE • 1 Nov 2019 • Vol 366, Issue 6465 • pp. 606-612 • DOI: 10.1126/science.aaw9285



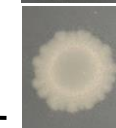
Flavobacterium sp. 98



12 + 1
candidates



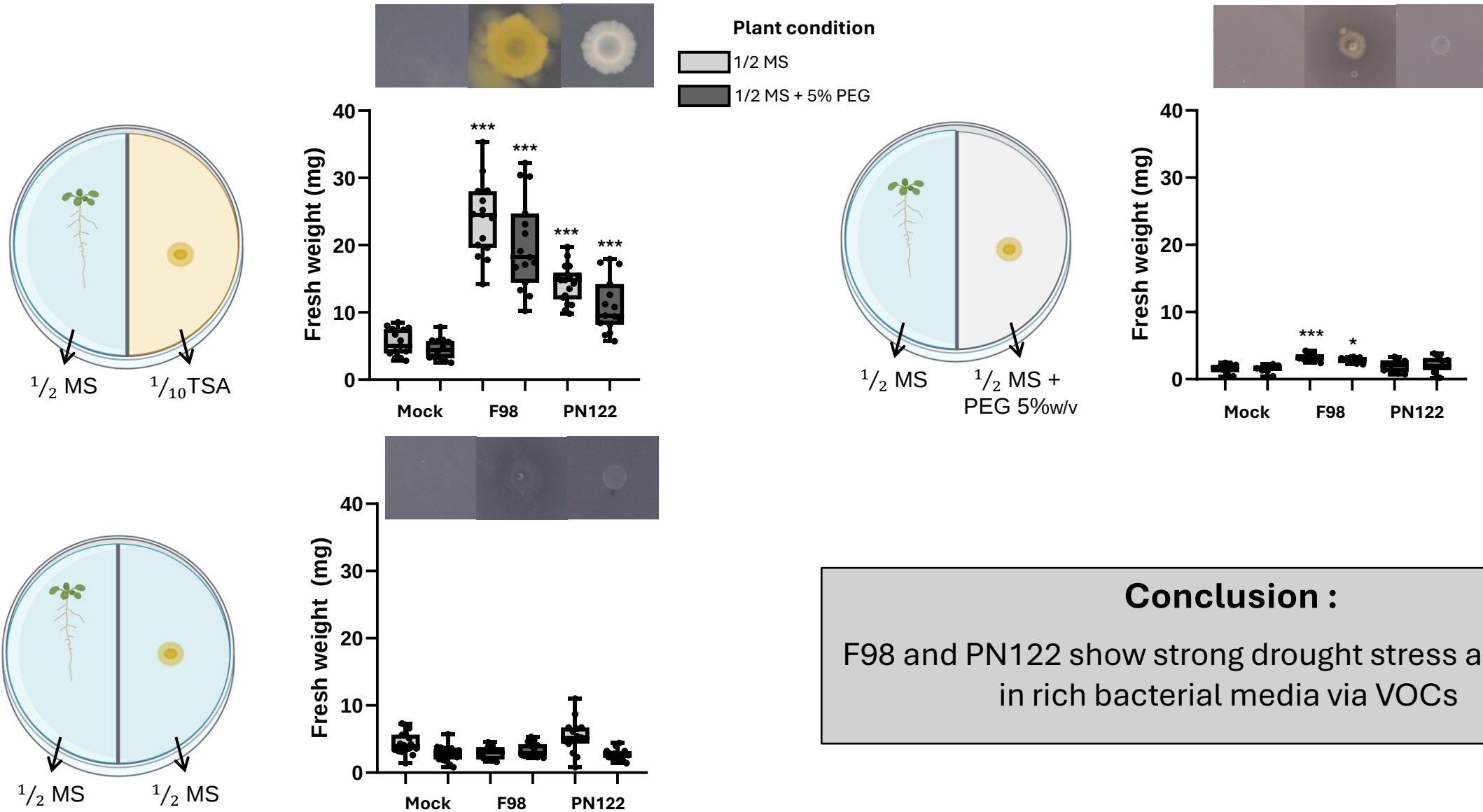
Flavobacterium
sp. 98 (F98)



Pseudomonas
sp. N122 (PN122)

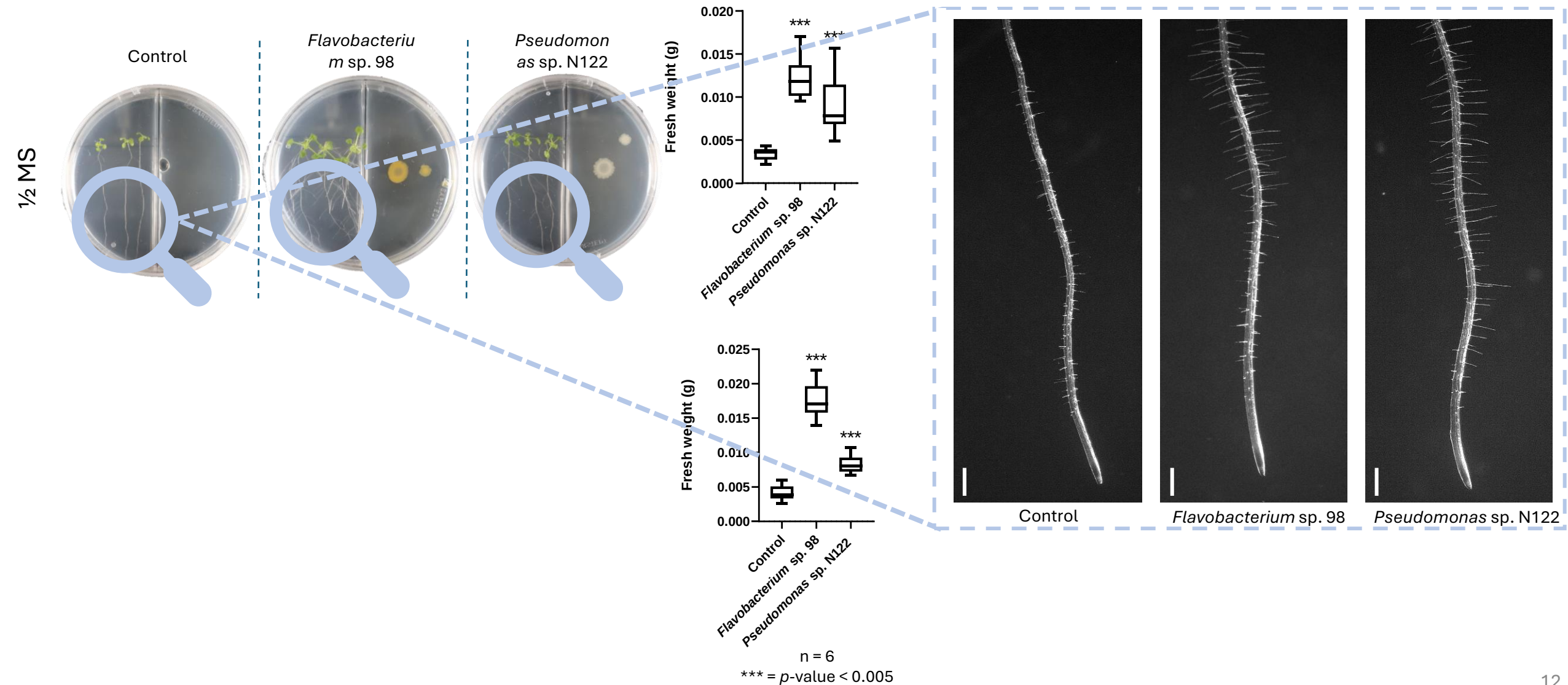
*Polyethylene glycol

Potential impact of VOCs on PGP and drought stress alleviation

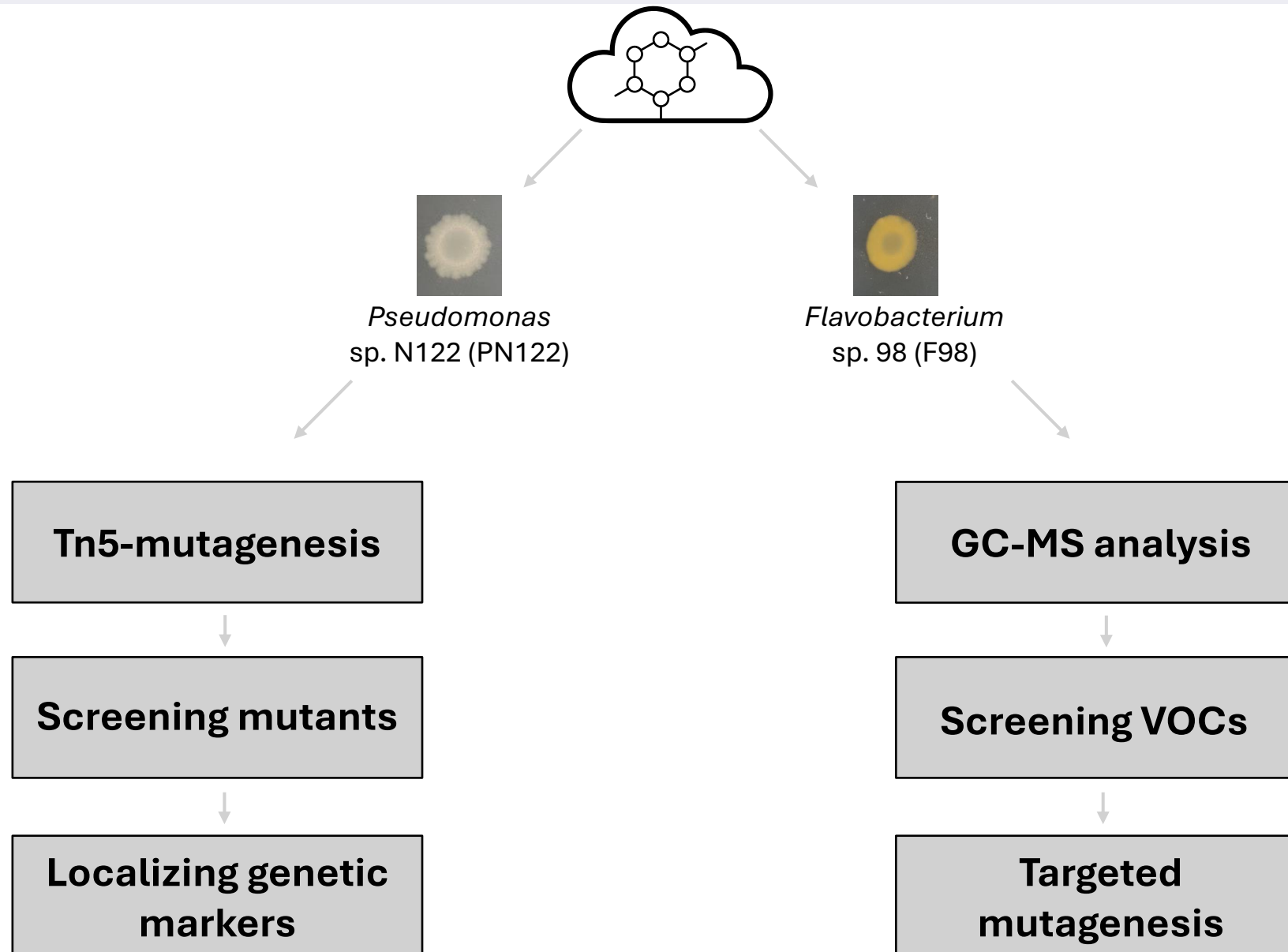


N ≥ 8, One-way ANOVA compared with relative mock treatment: *** = $p \leq 0.001$, * = $p \leq 0.05$, experiment was performed twice with similar results.

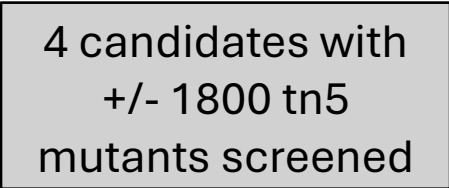
Drought stress alleviation via VOCs



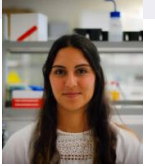
Strategies to unravel the underlying VOC(s)



Dr. Anjali Kulkarni is an Assistant Professor at the Department of Chemistry, University of California, San Diego. She received her Ph.D. from the University of California, San Diego, and completed her postdoctoral fellowship at the University of California, Berkeley. Her research interests include the synthesis of novel materials and their applications in catalysis and energy storage.

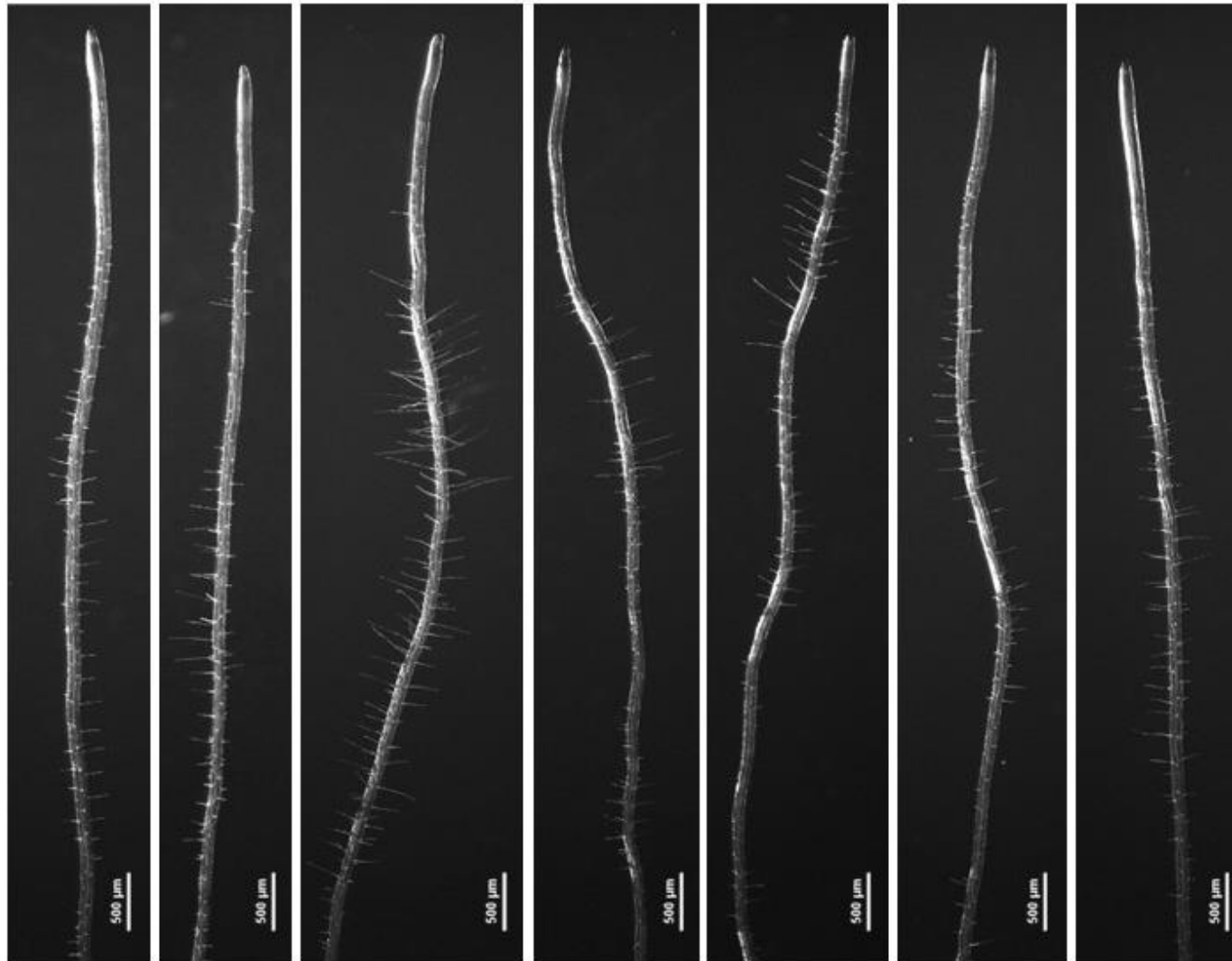


Random tn5 mutagenesis to target genetic markers



Cristina
Sarmiento

A



Control
(LB)

Control
(*E. coli*)

Ps. N122
(WT)

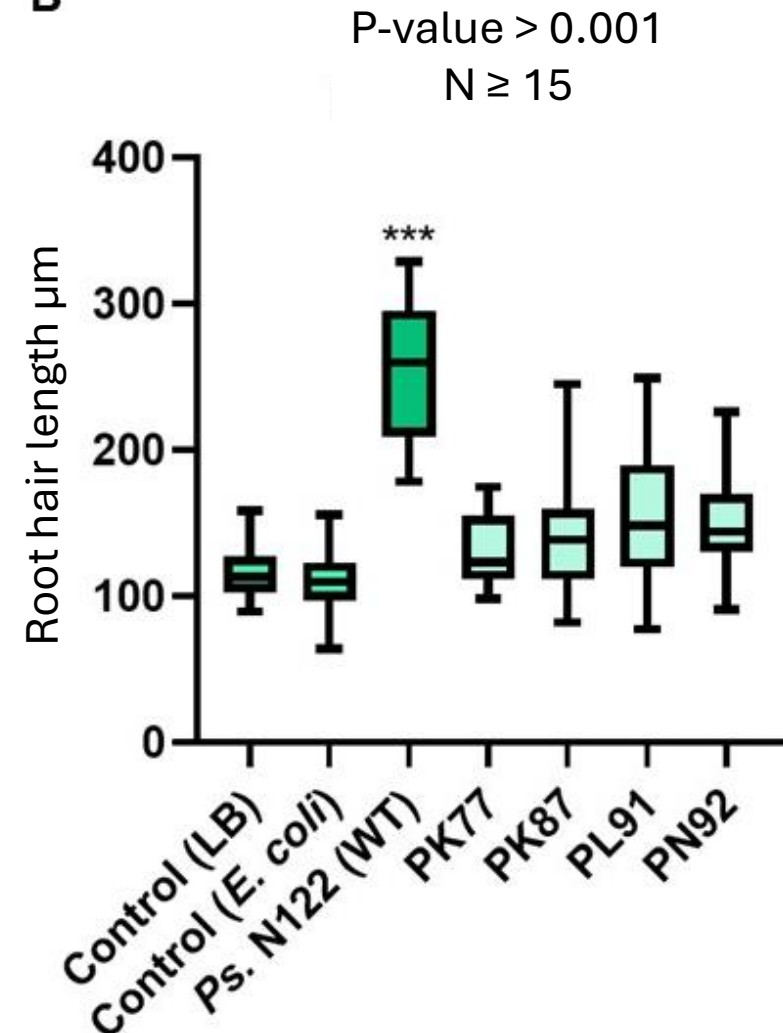
PL91

PN92

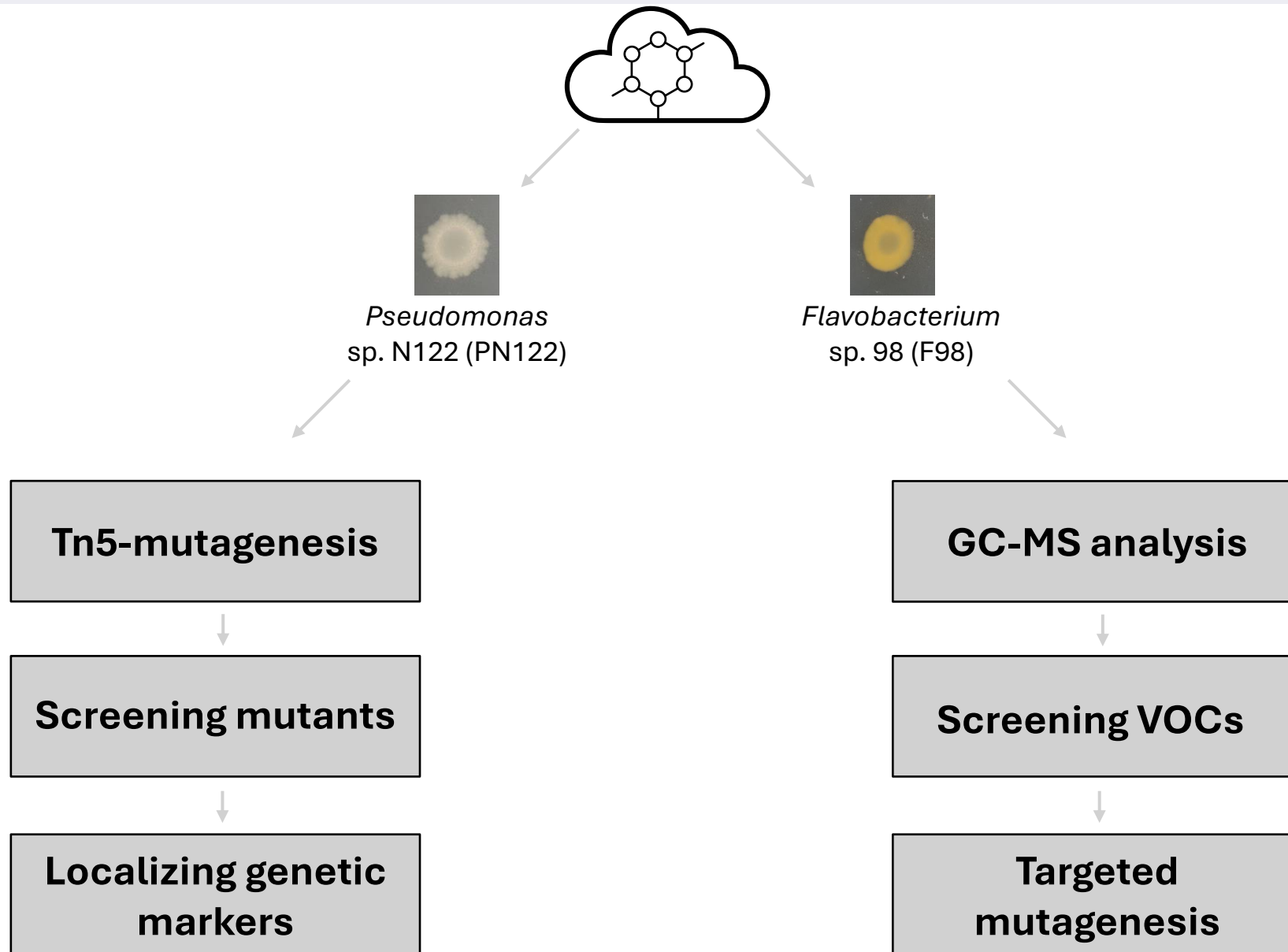
PK77

PK87

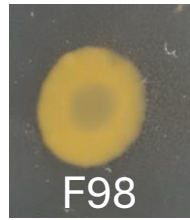
B



Strategies to unravel the underlying VOC(s)

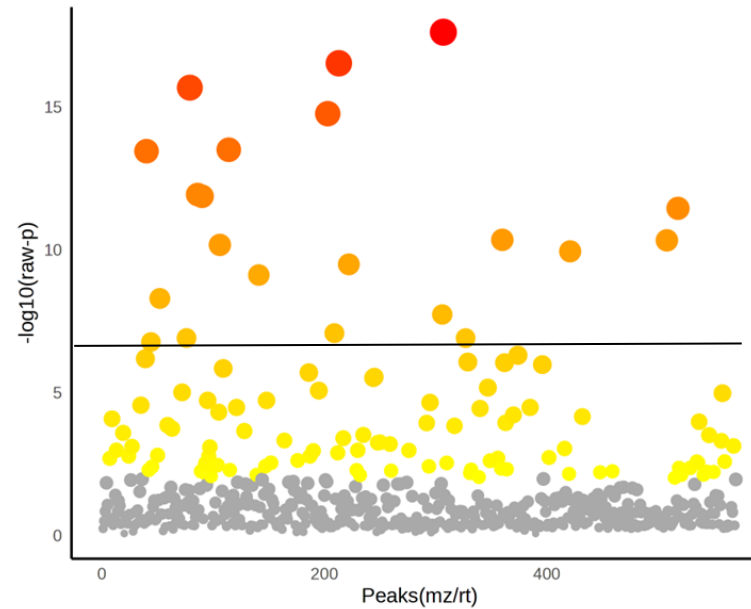


Mining the headspace of F98 and PN122 for VOCs

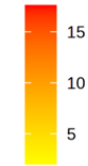


Agilent Technologies
MassHunter Workstation

MZmine 3



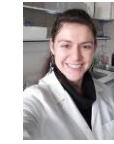
$-\log_{10}(\text{raw-p})$



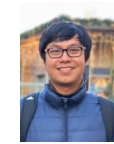
p.log



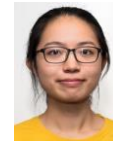
Ivana Staiano



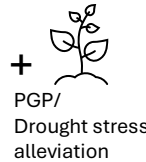
Dr. Christina Papazlatani



Muhammad Syamsu Rizaludin



Xinya Pan



25 Features*

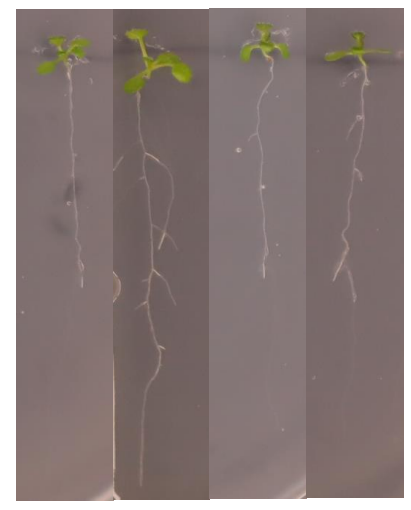
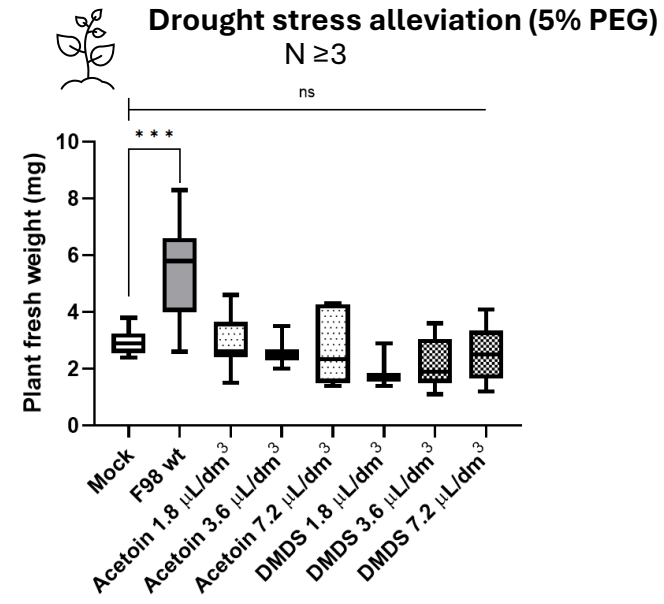
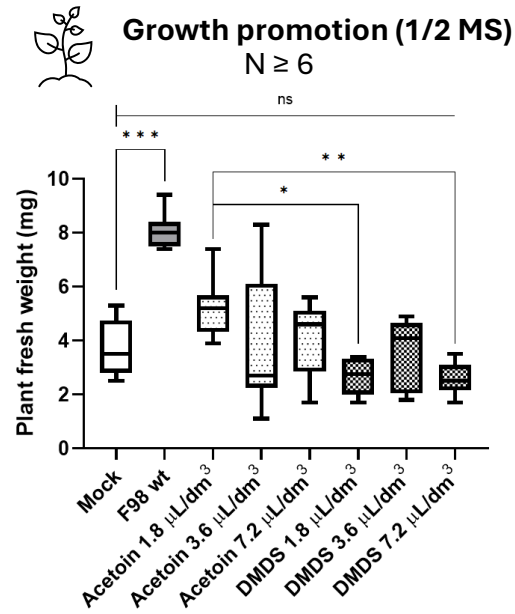
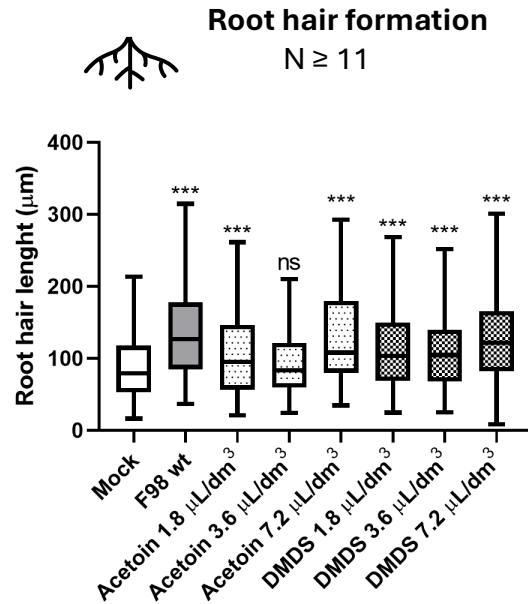
NIST
National Institute of Standards and Technology
U.S. Department of Commerce

22 VOCs

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



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



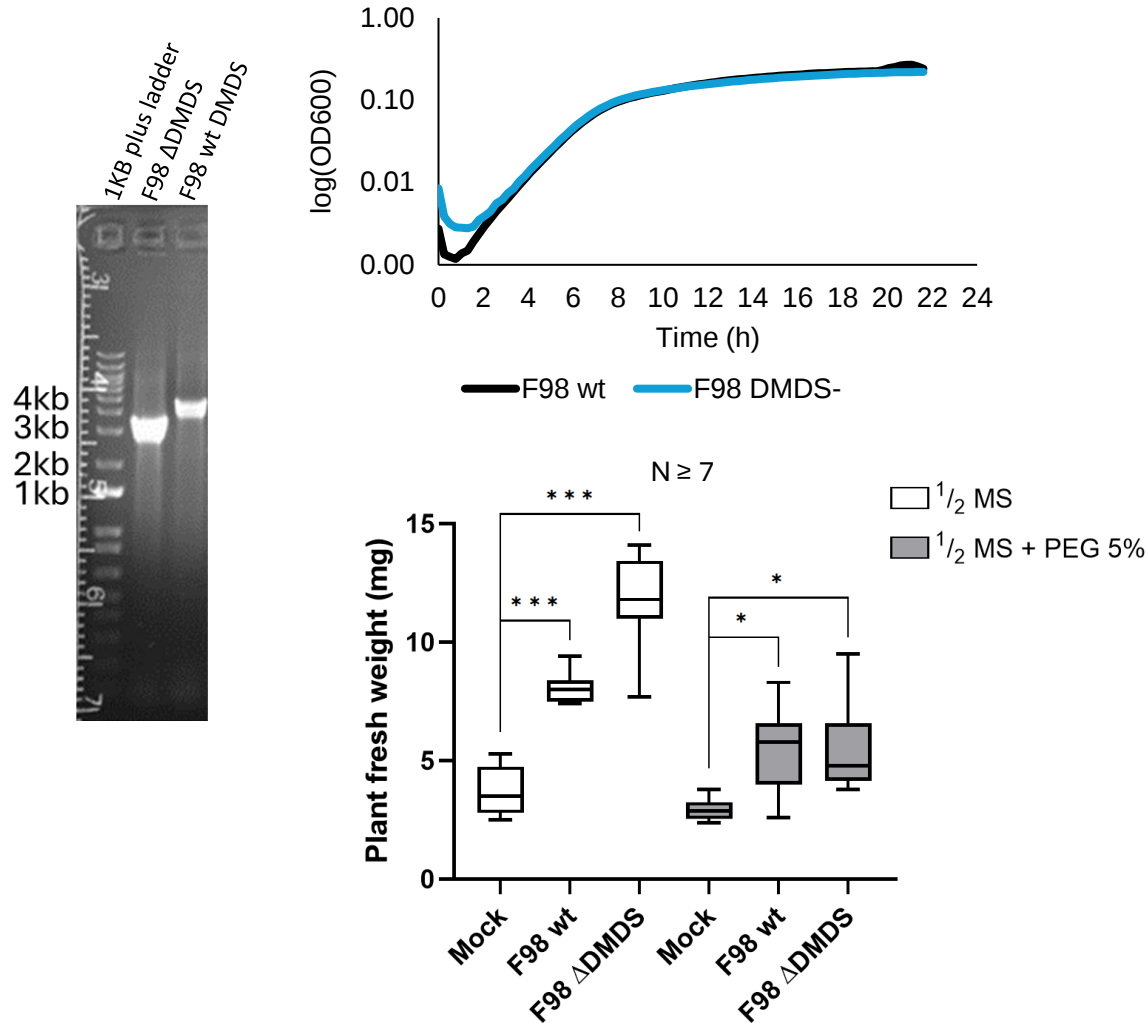
Conclusion :

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

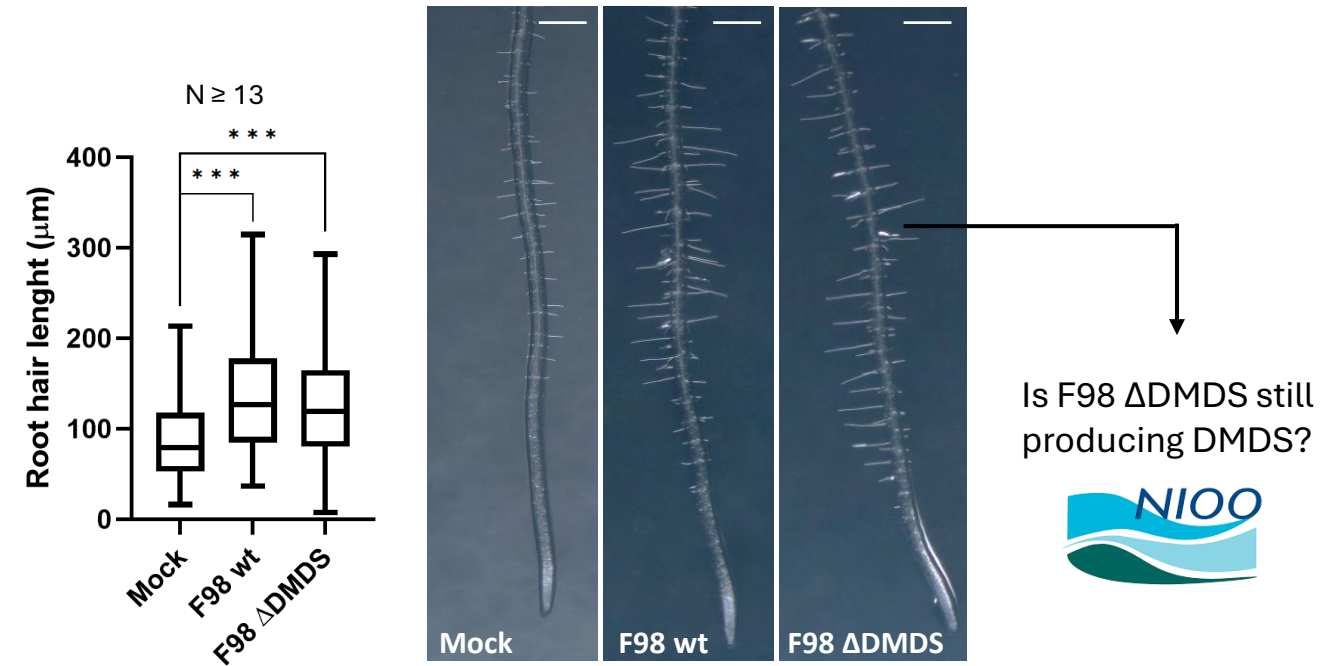
Mock F98 Acetoin DMDS

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

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*

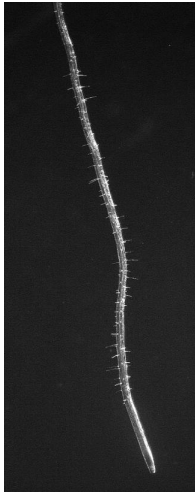


MicroRes : Microbial Induction of Plant Resilience to Drought Stress



Ivana Staiano

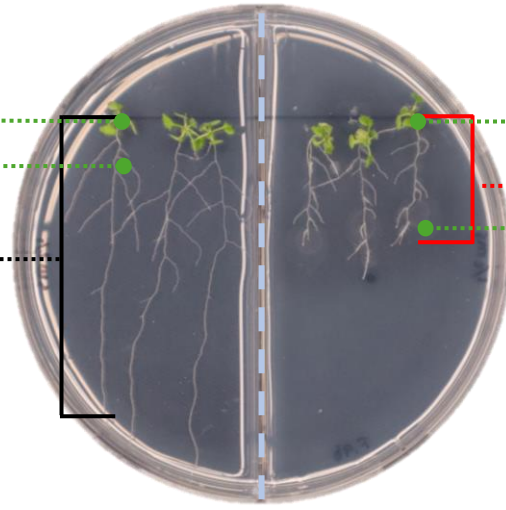
Control (MgSO₄)



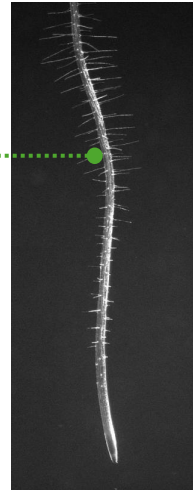
Volatile (F98)

Growth promotion
Root branching

Root length



Root hair length



Diffusible (F98)

Growth promotion

Root length

Root branching

Root hair length



Conclusion

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

Volatile

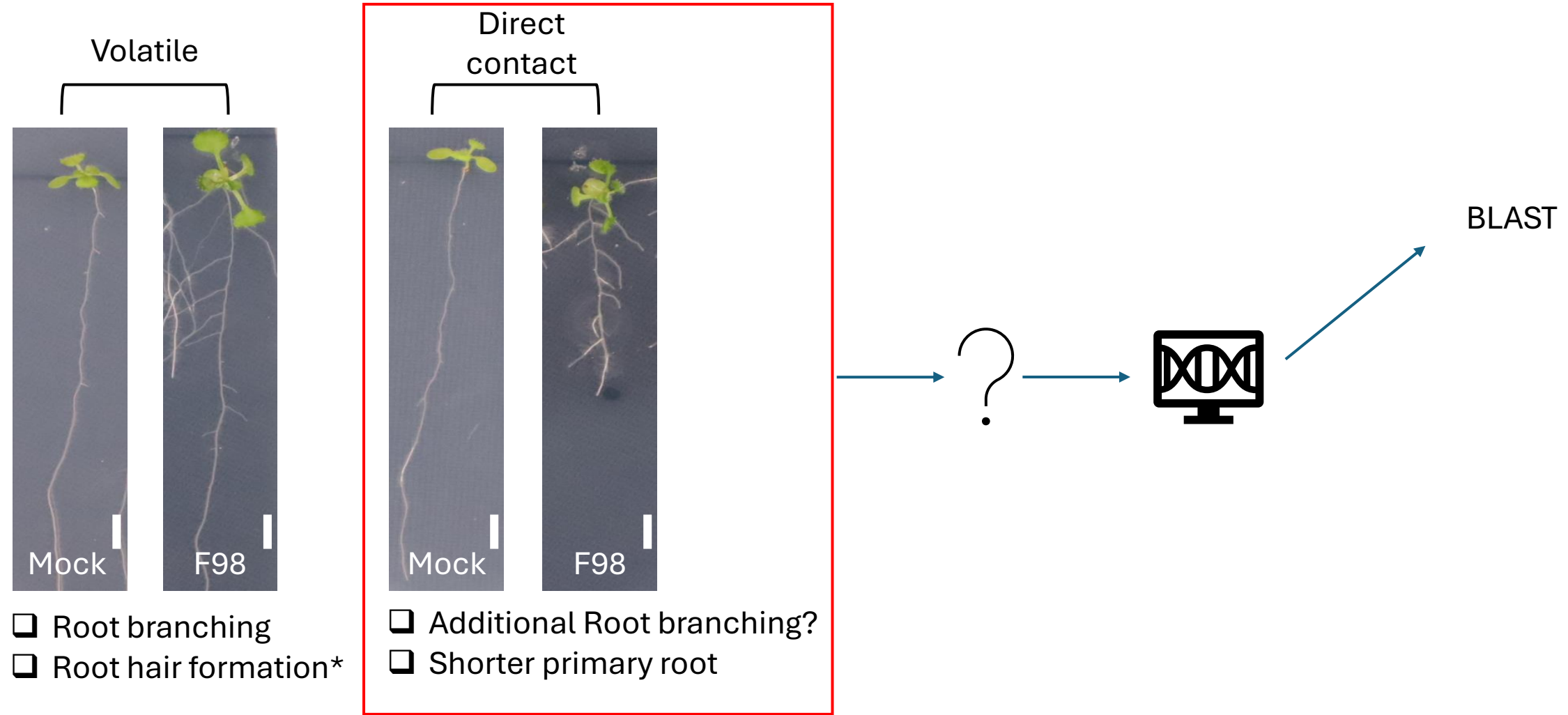
- + Growth promotion
- + Root branching
- + Root hair formation

Diffusible (contact)

- Root length

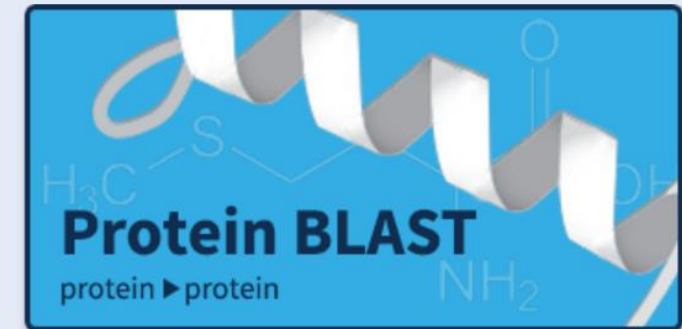
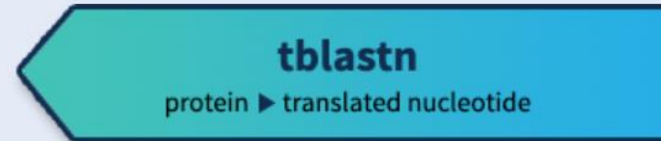
So we are dealing with multiple mechanisms!

Approaches to target candidate genes involved in our phenotype

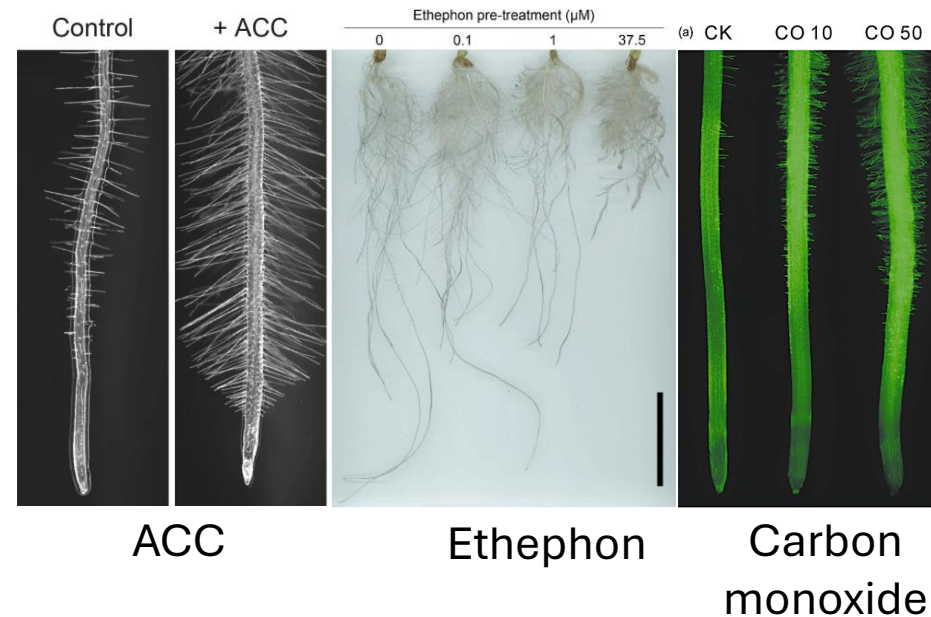


Literature research BLAST analysis in

Web BLAST

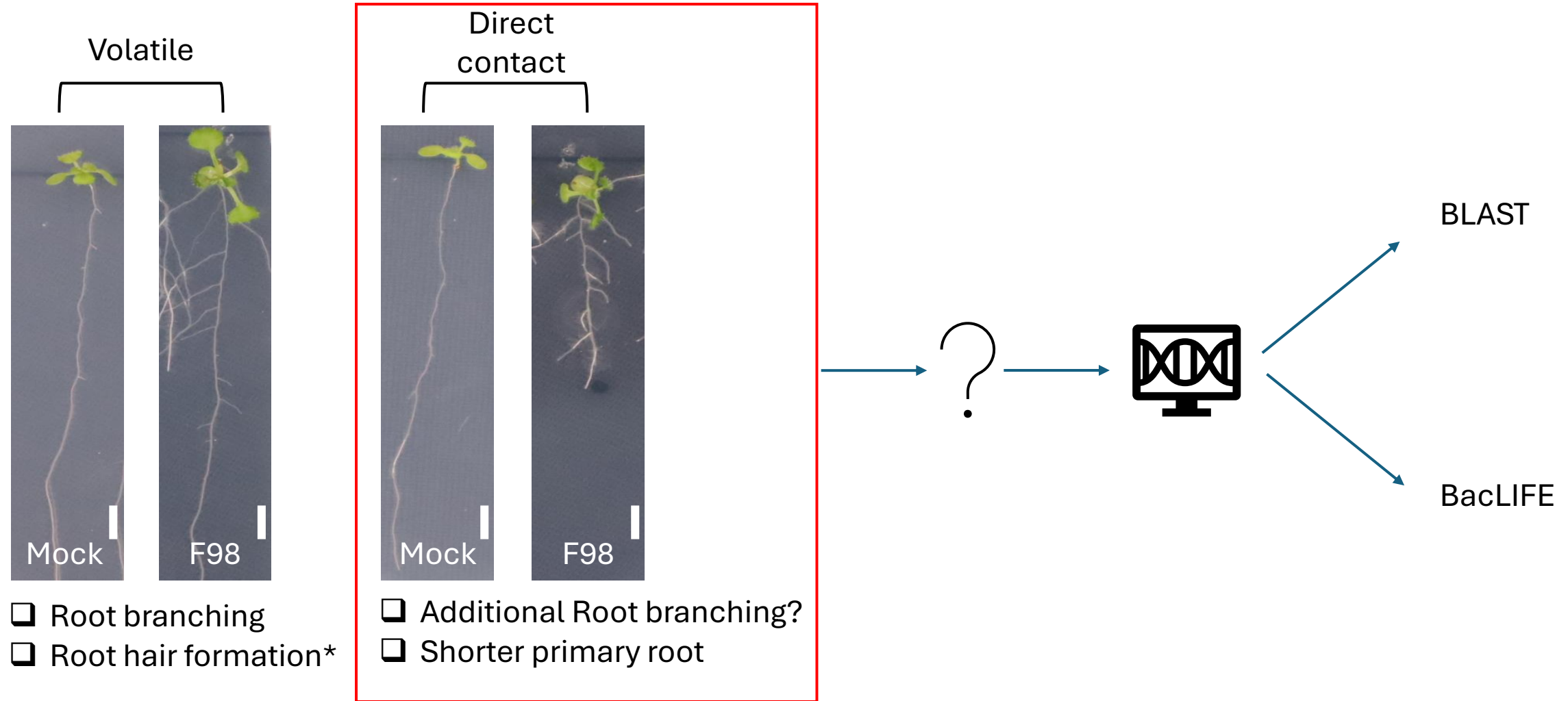


- Ethylene
- Acetylene
- 1-Aminocyclopropane-1-carboxylate (ACC)
- Ethephon
- Triplin
- Propylene
- carbon monoxide
- Isocyanides
- silver nitrate
- 1-methylcyclopropene (1-MCP)
- Jasmonic acid
- methyl jasmonate

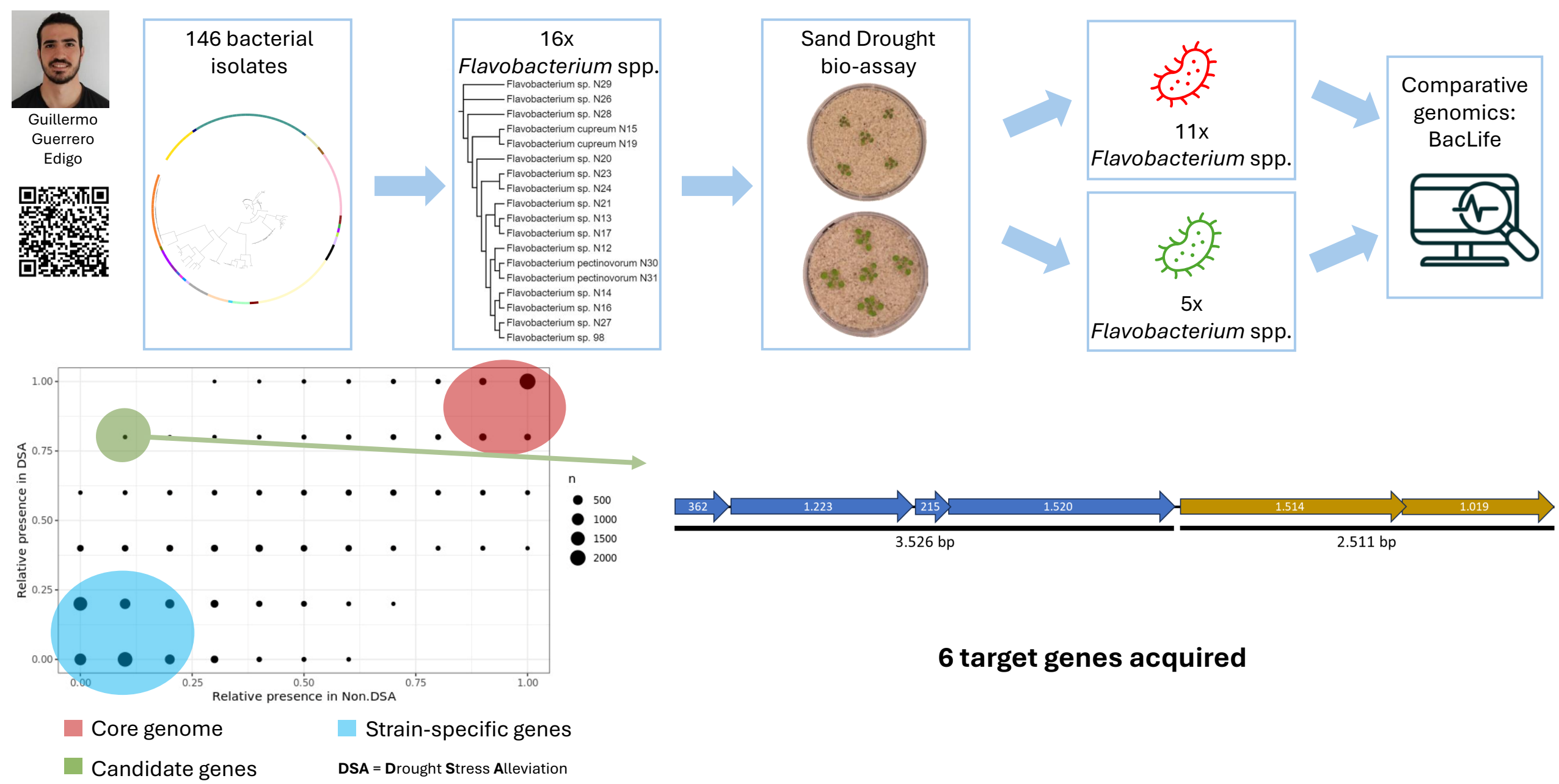


**Many target
genes acquired**

Approaches to target candidate genes involved in our phenotype



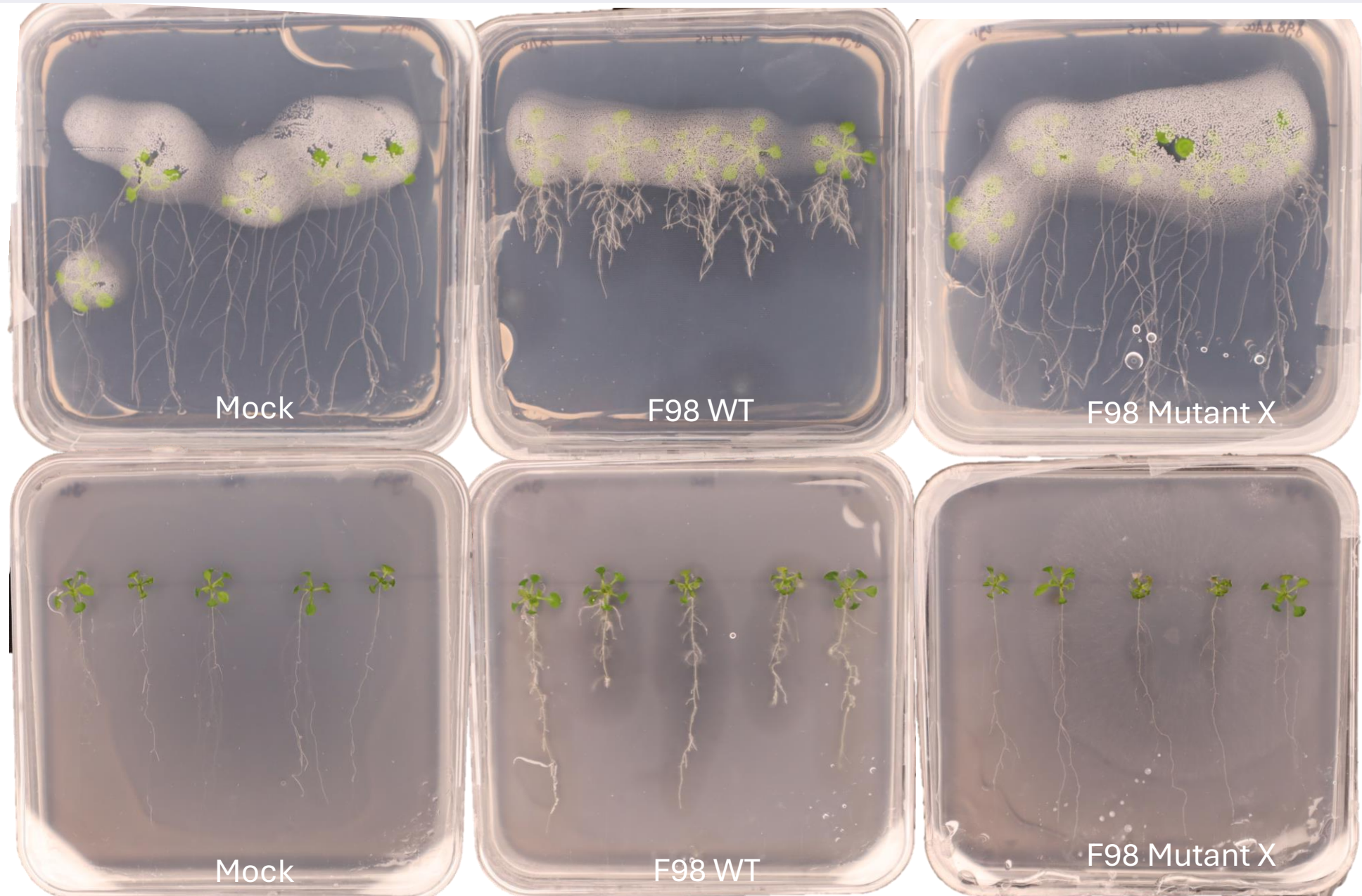
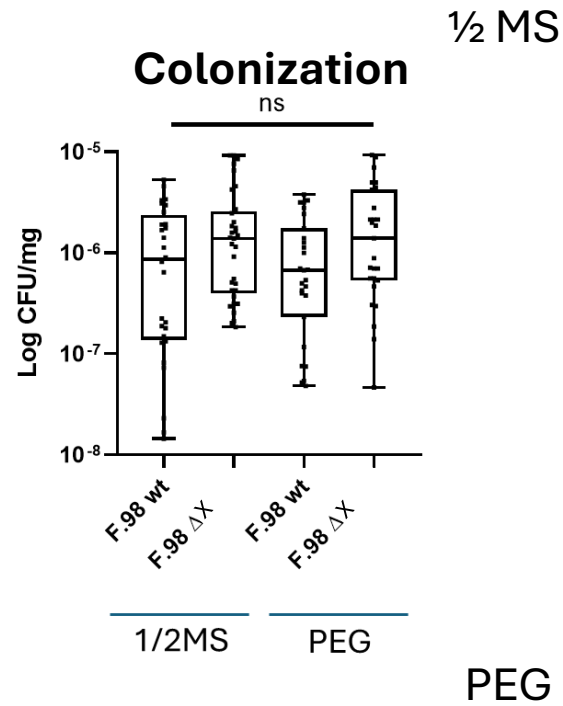
BacLIFE identify the genes involved in drought stress alleviation



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



Ivana Staiano



*Front view



Mock (MgSO_4)

F98 WT

F98 ΔX



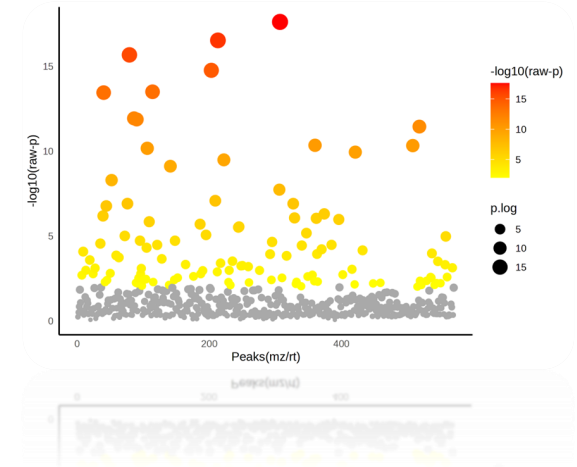
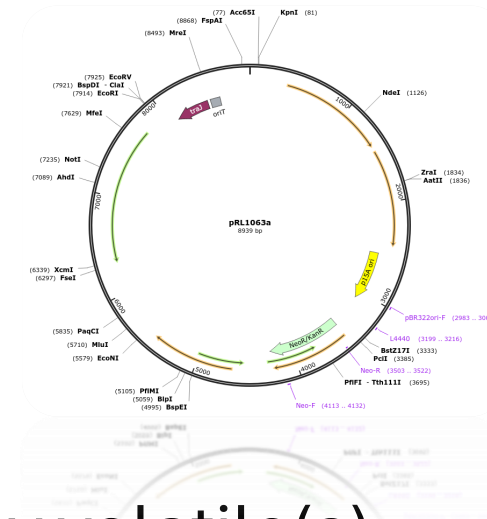
*Top view

Experiments are still on going!

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



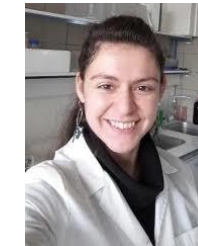
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