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Rhizobacterial volatile organic compounds modulate biomass production and root architecture in *Arabidopsis thaliana* (L.) Heynh. and *Brachypodium distachyon* (L.) P. Beauv.

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Introduction and Objectives

The ability of plants to take up water and mineral nutrients from the soil depends on their capacity to develop an extensive root system and on the interactions of roots with their soil environment, the rhizosphere. Studies of the mechanisms involved in the communication between plant growth-promoting soil micro-organisms and the root system is expected to lead to improved fertility management strategies. Up to now, the characterization of such interactions has been mainly focused on liquid diffusates but it has been recently reported that volatile organic compounds (VOC) also play a role as chemical messengers in positive interactions occurring in the rhizosphere and involving plants, bacteria, fungi and insects [1-2]. In this context, this project aims to better understand the ecophysiology of the rhizosphere of *Arabidopsis thaliana* Col-0 and *Brachypodium distachyon* 21-1, considering the interactions of both model plants with 19 bacterial strains.

Materials and Methods

Screening conditions

- Surface-sterilized and vernalized seeds and caryopses were co-cultivated with rhizobacteria for 10 days after inoculation of the bacterial compartment with 2 * 10^6 colony forming units of each of the 19 strains
- Plants were cultivated in a growth chamber [22°C, 65% RH, 20h/4h (D/N), 95 µmol·m^{-2} ·s^{-1} (LED lighting)]

Growth parameters

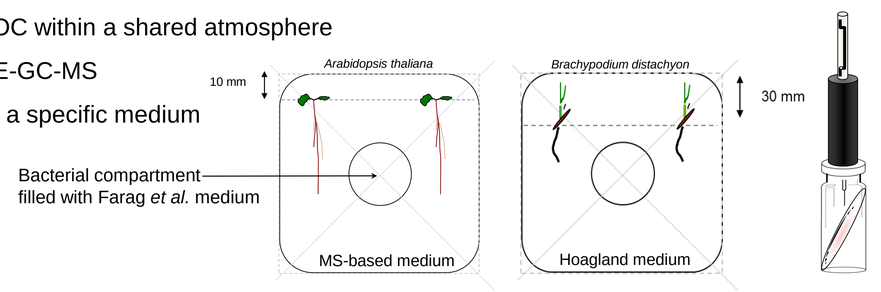
- Total fresh biomass, shoot and root biomass, root to shoot ratio
- Leaf area, developmental stage

Root architecture parameters (in progress)

- Primary root length, lateral and adventitious root numbers and cumulated lengths

Experimental set-up

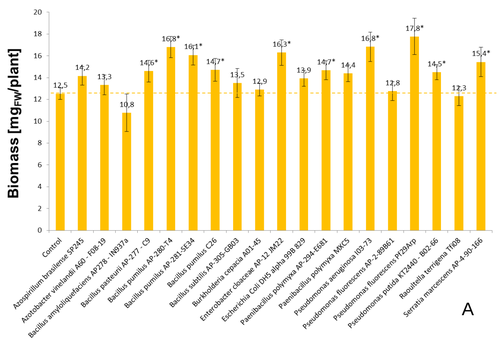
- *in vitro* cocultivation without physical contact between plants and bacteria
- Interactions through VOC within a shared atmosphere
- VOC analysis by SPME-GC-MS
- Each plant is grown on a specific medium

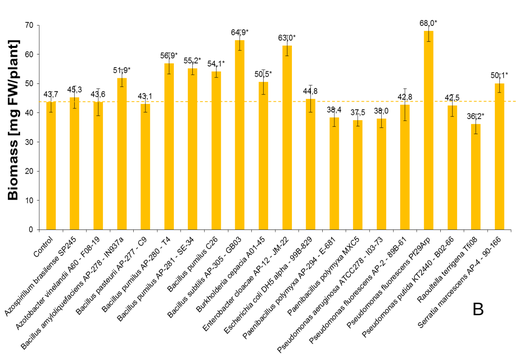


Results

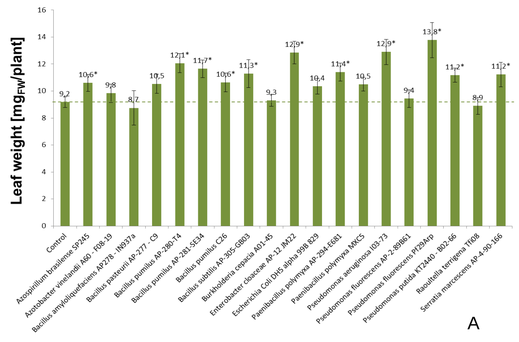
Volatile impacts on biomass production and partitioning

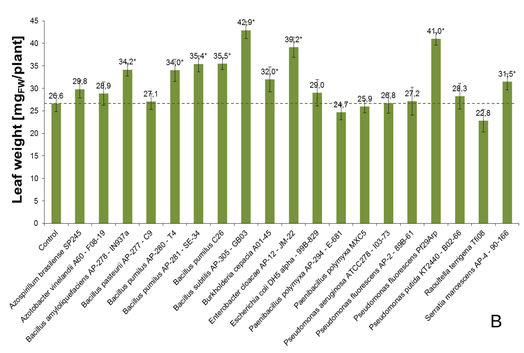
Total biomass



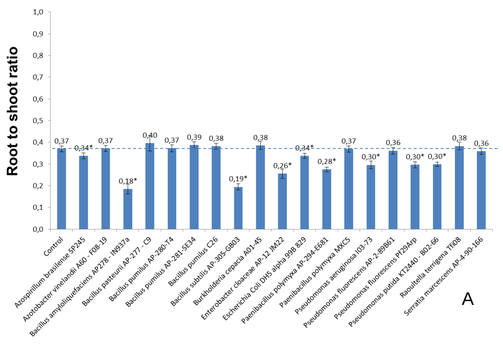


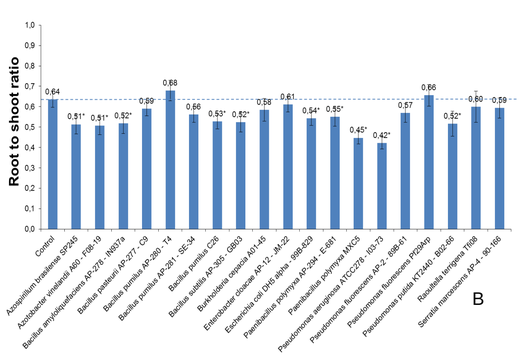
Shoot Biomass



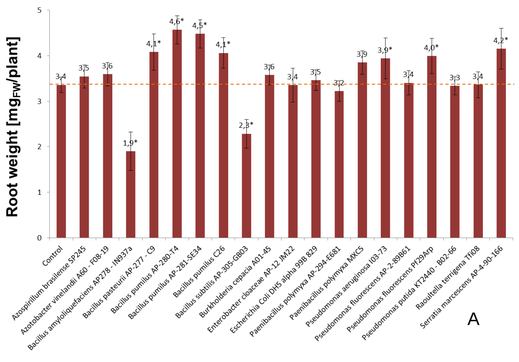


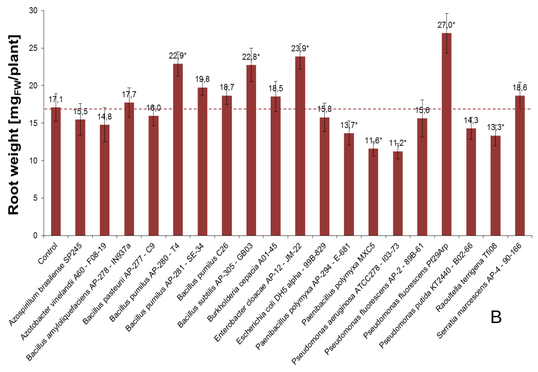
Root to shoot ratio





Root Biomass





Fresh biomass production and partitioning of *Arabidopsis* (A) and *Brachypodium* (B) plants grown in the presence of bacterial VOC for 10 days after 3 and 1 day(s) of pre-germination respectively. The presented data are means of four biological replicates (A) and one biological replicate (preliminary data, B), each replicate containing 16 plantlets. The error bars represent the confidence interval (α = 5%) and the * indicates a value which is significantly different from the control according to the Dunnett's test.

Volatile Impacts on root architecture

A

Bacterial strains	Arabidopsis TB	Arabidopsis L	Arabidopsis R	Arabidopsis RS	Brachypodium TB	Brachypodium L	Brachypodium R	Brachypodium RS
Azospirillum brasilense SP245		+		-				-
Azotobacter vinelandii A60 - F08-19								-
***Bacillus amyloliquefaciens* AP-278 - IN937a**			-	-	+	+		-
***Bacillus pasteurii* AP-277 - C9**	+		+					
***Bacillus pumilus* AP-280 - T4**	+	+	+	+	+	+	+	+
***Bacillus pumilus* AP-281 - SE-34**	+	+	+	-	+	+		
***Bacillus pumilus* C26**	+	+	+		+	+		-
***Bacillus subtilis* AP-305 - GB03**		+	-	-	+	+	+	-
***Burkholderia cepacia* A01-45**					+	+		
***Enterobacter cloacae* AP-12 - JM-22**	+	+		-	+	+	+	+
Escherichia coli DH5 alpha - 99B-829				-				-
***Paenibacillus polymyxa* AP-294 - E-681**	+	+		-			-	-
Paenibacillus polymyxa MXC5							-	-
***Pseudomonas aeruginosa* ATCC278 - I03-73**	+	+	+	-			-	-
Pseudomonas fluorescens AP-2 - 89B-61								
***Pseudomonas fluorescens* Pf29Arp**	+	+	+	-	+	+	+	+
***Pseudomonas putida* KT2440 - B02-66**	+	+		-				-
Raoultella terrigena TfI08					-		-	
***Serratia marcescens* AP-4 - 90-166**	+	+	+		+	+		

B

Rhizobacterial VOC impacts on biomass (A) and *Brachypodium* root architecture (B). Colour code indicates the grouping of strains based on plant responses.

Rhizobacterial volatile measurements

Semi-quantitative rhizobacterial VOC measurements (n = 4 +/- MSE) in 12 ml headspace (HS) after 24 hours of growth at 37°C on Farag *et al* (2006) medium. A triple DVB/CAR/PDMS fiber was selected for the analysis using methyl-octanoate as internal standard.

Conclusions and perspectives

- In our screening conditions, 10 and 9 bacterial strains promote total biomass production in *Arabidopsis* and *Brachypodium* respectively. Some strains promote plant growth for only one out of the two species.
- For each model plant, four groups of bacterial strains can be identified based on their effects on biomass production and partitioning.
- The growth promotion effects can be linked to modifications in shoot development (leaf plastochron index) and root architecture (induction of branching and adventitious root production). The quantitative assessment of root architecture changes using EZ-Rhizo software is in progress.
- The plant growth-promoting strains emit different volatile blends that should be further investigated (via principal component analysis) to be linked to their biological effects. Therefore, further work is needed to investigate the whole datasets to raise new scientific hypothesis.
- *Enterobacter cloacae* AP12-JM22 and *Serratia marcescens* AP4-90-166 promote plant growth without producing 2,3-butanediol which is a well-known VOC promoting *Arabidopsis* growth.
- Dose effects and interactions with mineral availability will be investigated on a restricted subset of bacteria using contrasted plant genotypes.

References

1. Ryu et al. 2003. Proc Natl Acad Sci USA 8: 4927-4932.
2. Kai et al. 2007. Arch Microbiol 187: 351-360.
3. Farag et al. 2006. Phytochemistry 67: 2262-2268.

Acknowledgements

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