Root2Res

Root phenotyping and genetic improvement for
rotational crops resilient to environmental change

Plasticity of traits and its use to study rhizosphere

Mid-Term symposium, ESA Congress, Rennes 29/08/2024

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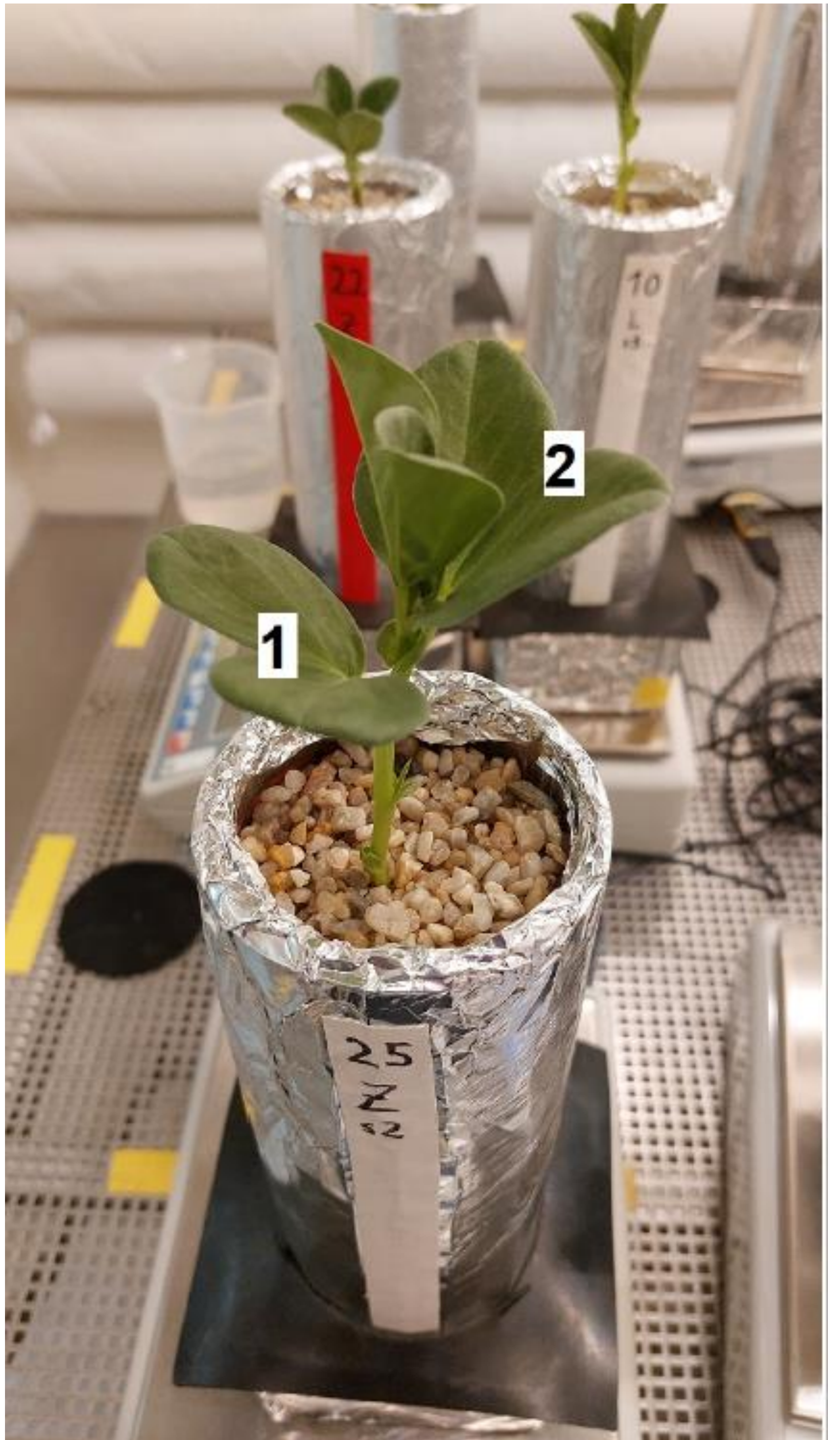
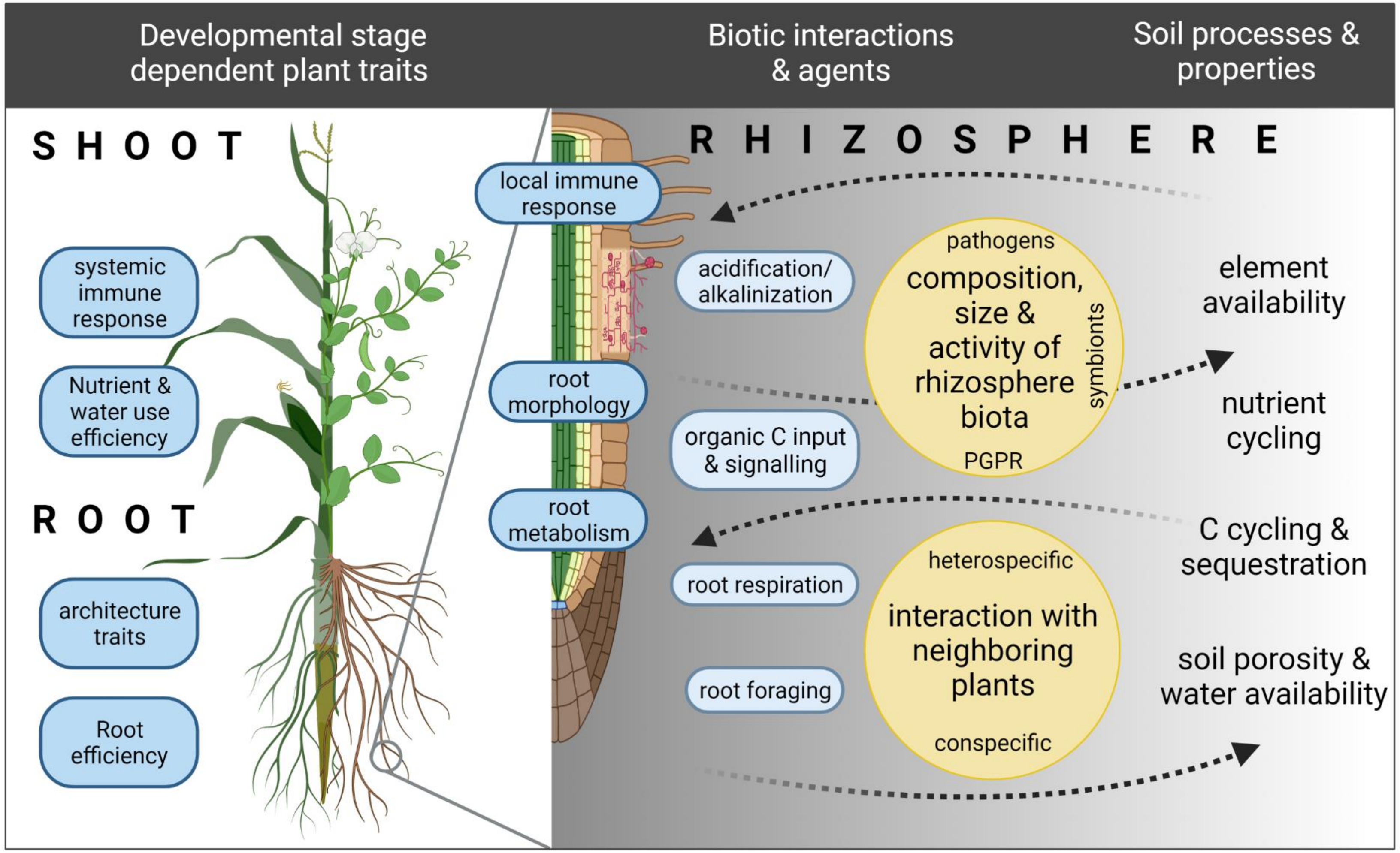


Root2Res approach: rhizosphere “extended phenotype”



Oburger et al. 2022, Plant and Soil

Architectural traits

Exudates

Microbiome activities

Traits related to

Soil exploration

Soil resources exploitation

Interactions with soil microbiome

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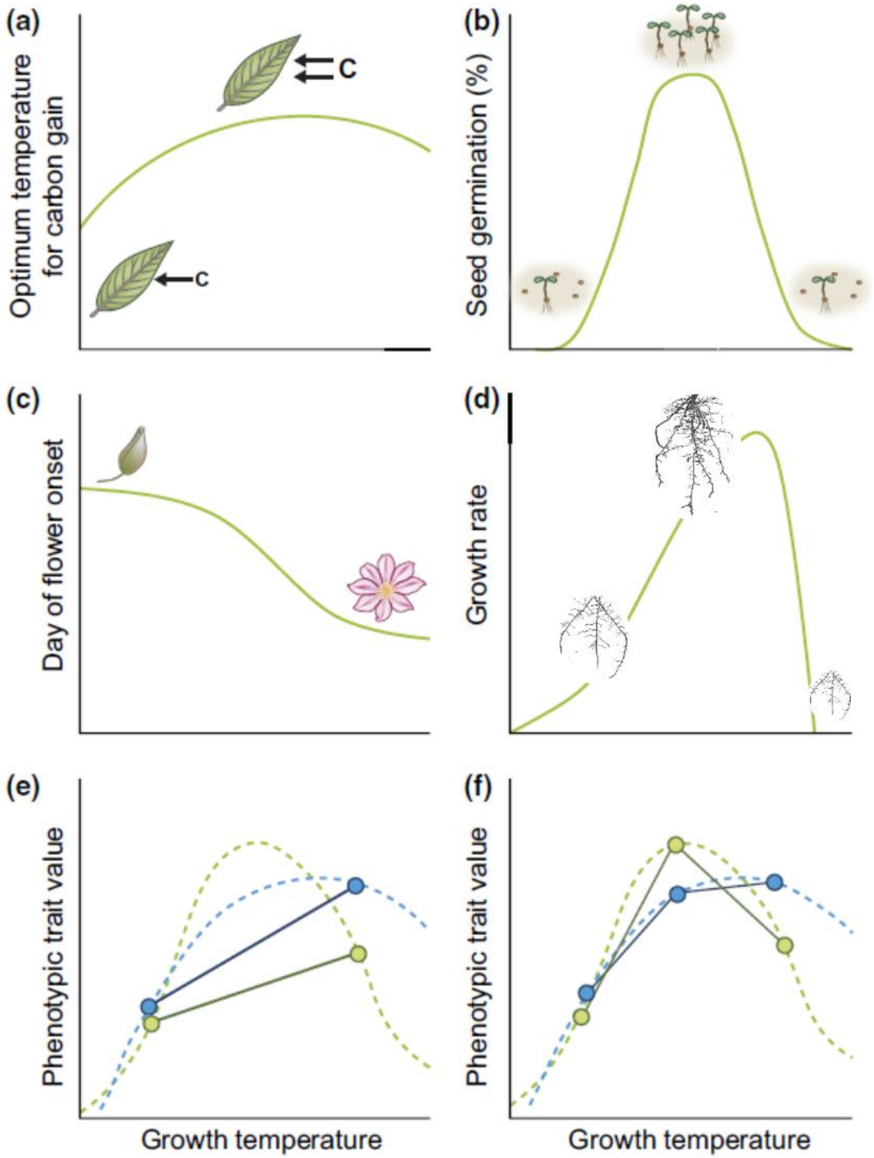
Plasticity : a definition

Definition of phenotypic plasticity

Phenotypic plasticity is the ability of an organism to alter its phenotype in response to the environment (Sultan 2000) and may involve changes in physiology, morphology, anatomy, development, resource allocation, or mutualistic interactions with the microbiome.

Care should be taken in deciphering between the genetic variation within a species resulting in phenotypic plasticity and the phenotypic plasticity of an individual genotype (Valladares et al. 2006 and citations therein)

Quantification of plasticity



Traditionally reaction norms of different traits can be investigated by directly comparing the response of the trait to an environment by calculating and comparing the slopes or coefficients of variation (CV).

However, these can become difficult to compare when reaction norms are non-linear or traits for comparison do not follow similar patterns of reaction norm (Fig. modified from Arnold et al. 2019).

Quantification of plasticity – Relative distance plasticity index (RDPI)

Valladares et al. 2006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Practice Abstract

How to calculate root phenotypic plasticity as a trait for crop breeding programs

https://root2res.eu/

The index is the average of the absolute pairwise distances between observations where environmental treatment differs.

i denotes the individual observation
j denotes the environment treatment level

Pairwise distances

$$d_{ij} \rightarrow i'j' = \left| x_{ij} - x_{i'j'} \right|$$

Distances are standardised by the sum of the pair, which gives values between 0 and 1

Finally, the relative distance plasticity index (RDPI) can be calculated as:

$$RDPI = \frac{\sum \left(\frac{\left| d_{ij} \rightarrow i'j' \right|}{x_{ij} + x_{i'j'}} \right)}{n}$$

Where *n* is the total number of pairwise distances

- No linear reaction norm is required
- Due to standardization shape of reaction norms of different factors of environment can be neglected
- No control treatment required as reference

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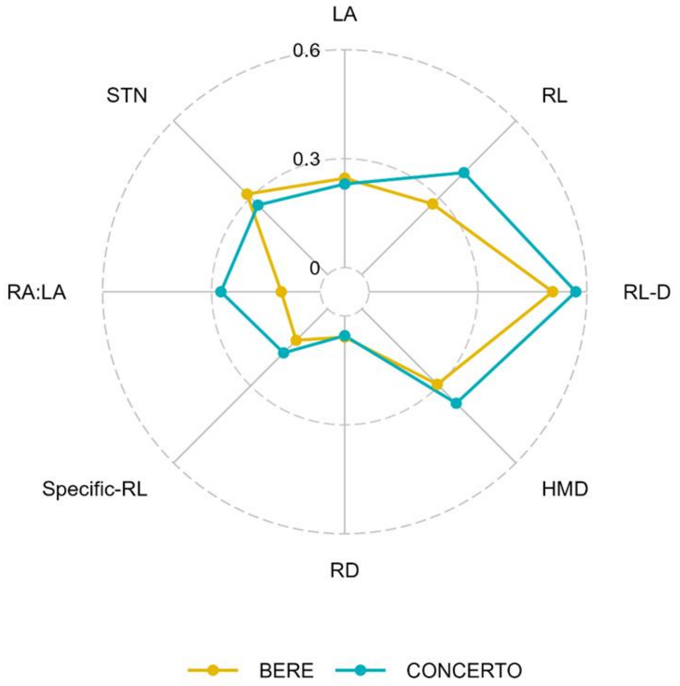
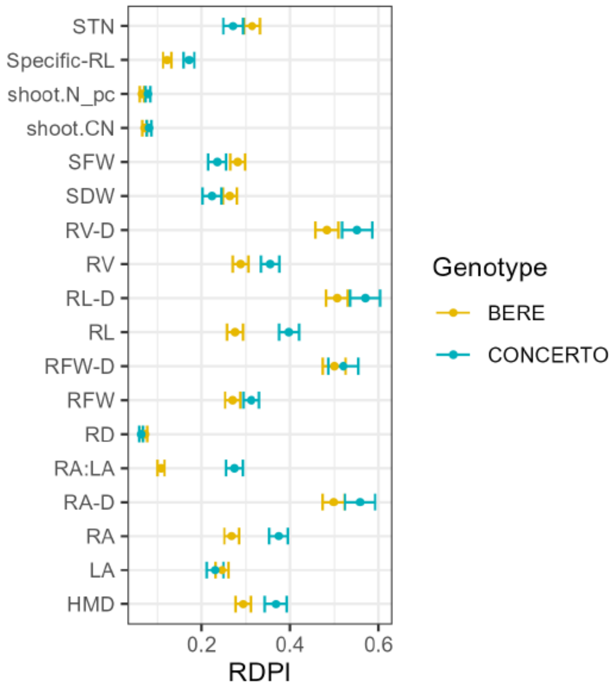
Quantification of plasticity – RDPI – graphical representation of results





- At a glance the estimated RDPI can be visualized for a number of traits using spider web diagrams
- Standardizing which parameters are presented and in which sequence conveys the information quickly
- Information on shoot traits can be included (here leaf area LA)
- Approach can be used by breeding companies
- Estimated RDPI could also be visualized in a dot chart with associated SEM.

CE experiments comparing 2 barley genotypes under contrasted environment (UFZ - Doris Vetterlein, Jessica Clayton and Sebastian Blaser, 2023).
LA: leaf area in cm2, RA: root surface area in cm2, RL: root length in cm, RL-D: root length density in cm/cm3, HMD: root half mean distance in cm, RD: root diameter in mm, Specific-RL: specific root length in cm/g, RA:LA: root:shoot surface area ratio and STN: shoot total nitrogen in mg.

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Quantification of plasticity – importance of environment definition

- Example of water availability

https://hortnews.com/wp-content/uploads/2023/11/Photo-credit-BNN-.jpg

https://s7d2.scene7.com/is/image/TWCNews/drought-dry-cracked-terrain1jpg

⇒ How to mimic these scenarios in lab experiment?
    ⇒ Timing
    ⇒ Intensity
    ⇒ Duration

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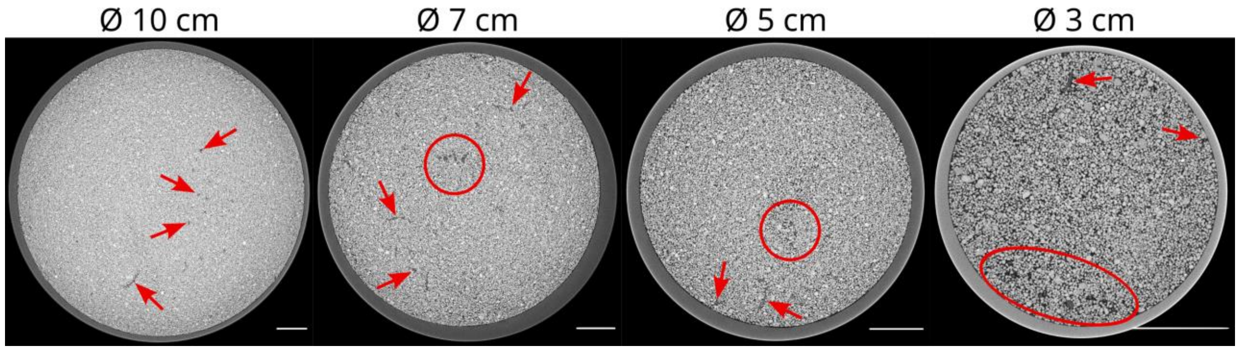
Case study "pot size experiment" – impact of controlled environment conditions on plasticity assessment



For X-ray CT there is a trade-off between resolution and sample size

RDPI is sensitive to the environmental range covered.

CE experiments comparing 2 barley genotypes under contrasted environment (UFZ - Doris Vetterlein, Jessica Clayton and Sebastian Blaser, 2023).
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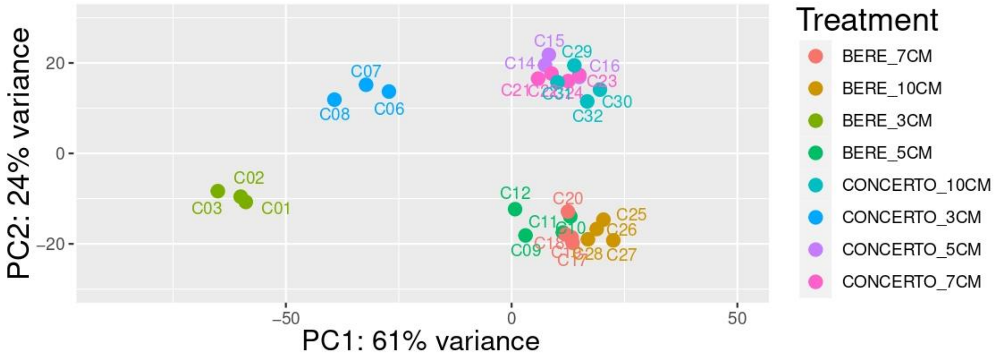
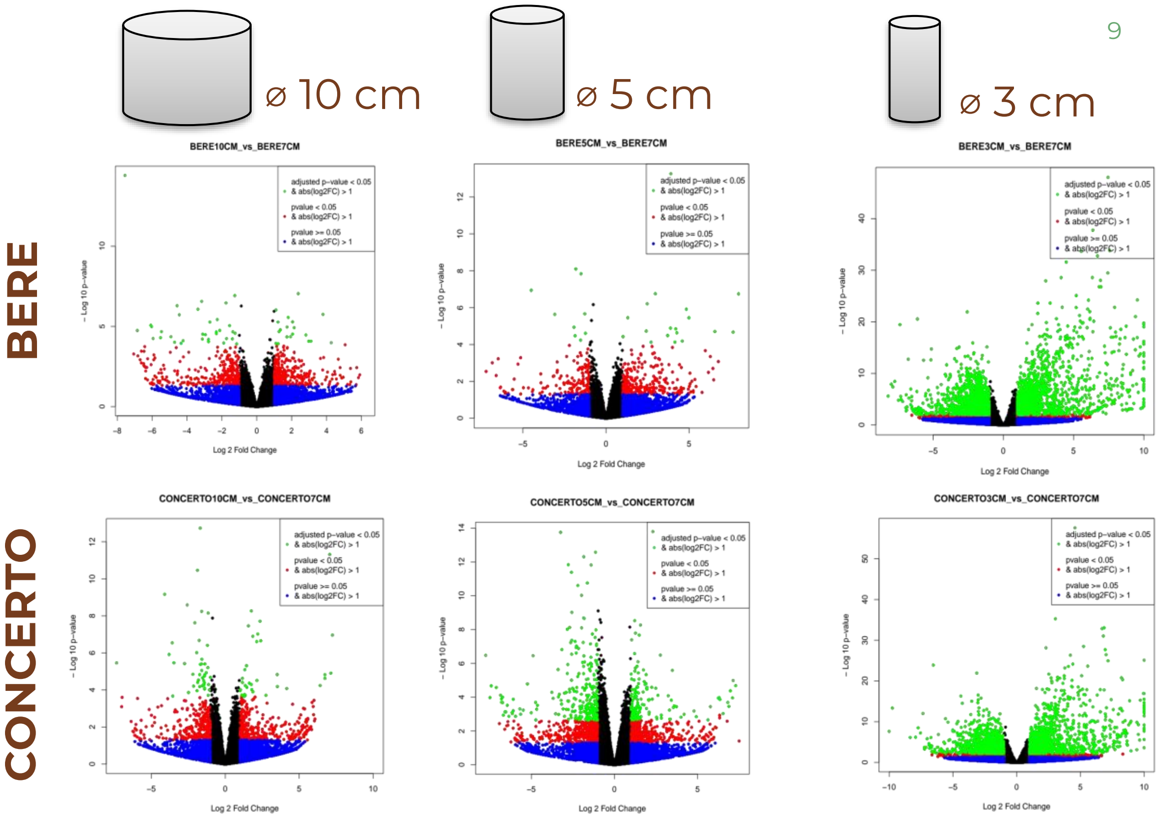
Case study “pot size experiment” – impact of controlled environment conditions on gene expression



- Clear clusters suggest distinct groups in gene expression data

- Genes are differentially expressed, in particular in the small pots of 3 cm ∅ (note: 7 cm ∅ serves as reference)



Volcano plots. The log2 FC indicates the mean expression level for each gene, and the -Log10 p-value indicates significance of the differences based on DESeq2 multiple pairwise comparison. Each dot represents one gene. **Left side of the plots showed down regulated genes, right part up-regulated ones.** Blue dots represent no significant DEGs between a condition group and control group with abs(log2FC>1), the red dots represent DEGs with abs(log2FC>1) with a p-value <0.05, and green dots represent DEGs with a padj <0.05.

⌀ 7 cm: CONTROL condition for DEG identification

CE experiments comparing 2 barley genotypes under contrasted environment
Transcriptomic analyses by CNR (Fabiano Sillo and Raffaella Balestrini 2023) based on samples from UFZ experiment (Doris Vetterlein, Jessica Clayton and Sebastian Blaser, 2023).

Root2Res First experiments on root exudates

JHI, BOKU

Objectives:

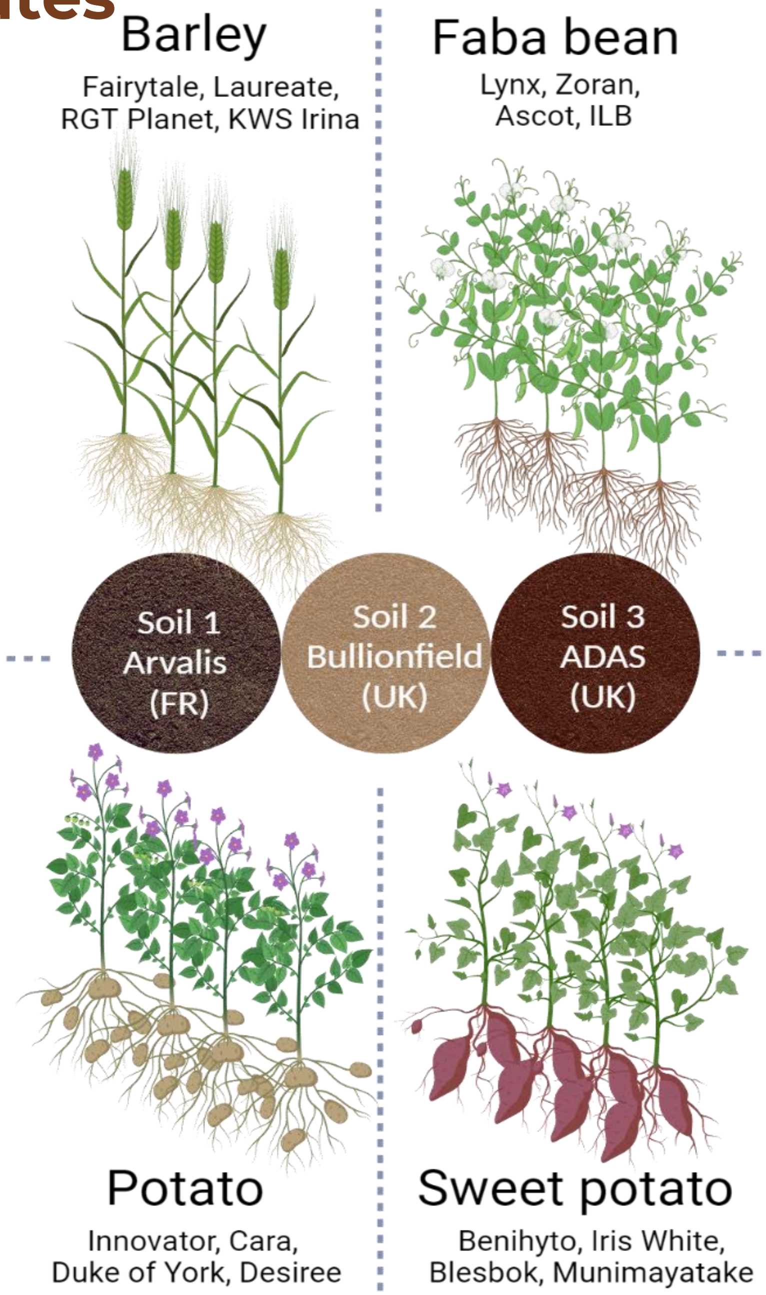
- Characterization of rhizosphere traits focusing on **root exudation & associated rhizosphere microbiome => Rhizosphere traits phenotyping tool box**

Challenge:

- Linking exudation to rhizobiome => Sampling root exudates and associated rhizosphere microbiome from the same plant

Centralized CE pot experiment at JHI

- with barley, Faba bean, potato, sweet potato
- 3 experimental soil
- 1 sampling time point
- Biomass & root morphology
- Joint exudate & rhizobiome sampling



Barley

Fairytale, Laureate, RGT Planet, KWS Irina

Faba bean

Lynx, Zoran, Ascot, ILB

Soil 1 Arvalis (FR)

Soil 2 Bullionfield (UK)

Soil 3 ADAS (UK)

Potato

Innovator, Cara, Duke of York, Desiree

Sweet potato

Benihyto, Iris White, Blesbok, Munimayatake

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Sampling exudates & rhizosphere soil from the same plant

① Removal off rooted soil block from pot

② Bulk soil removal by gentle rinsing

③ Collection of rhizosphere soil by dipping in fresh solution 1-2 times

④ Capturing potential cell damage & osmotic adjustment effects

⑤ Exudation sampling 2-3h

https://youtu.be/MM5MItbYgYI

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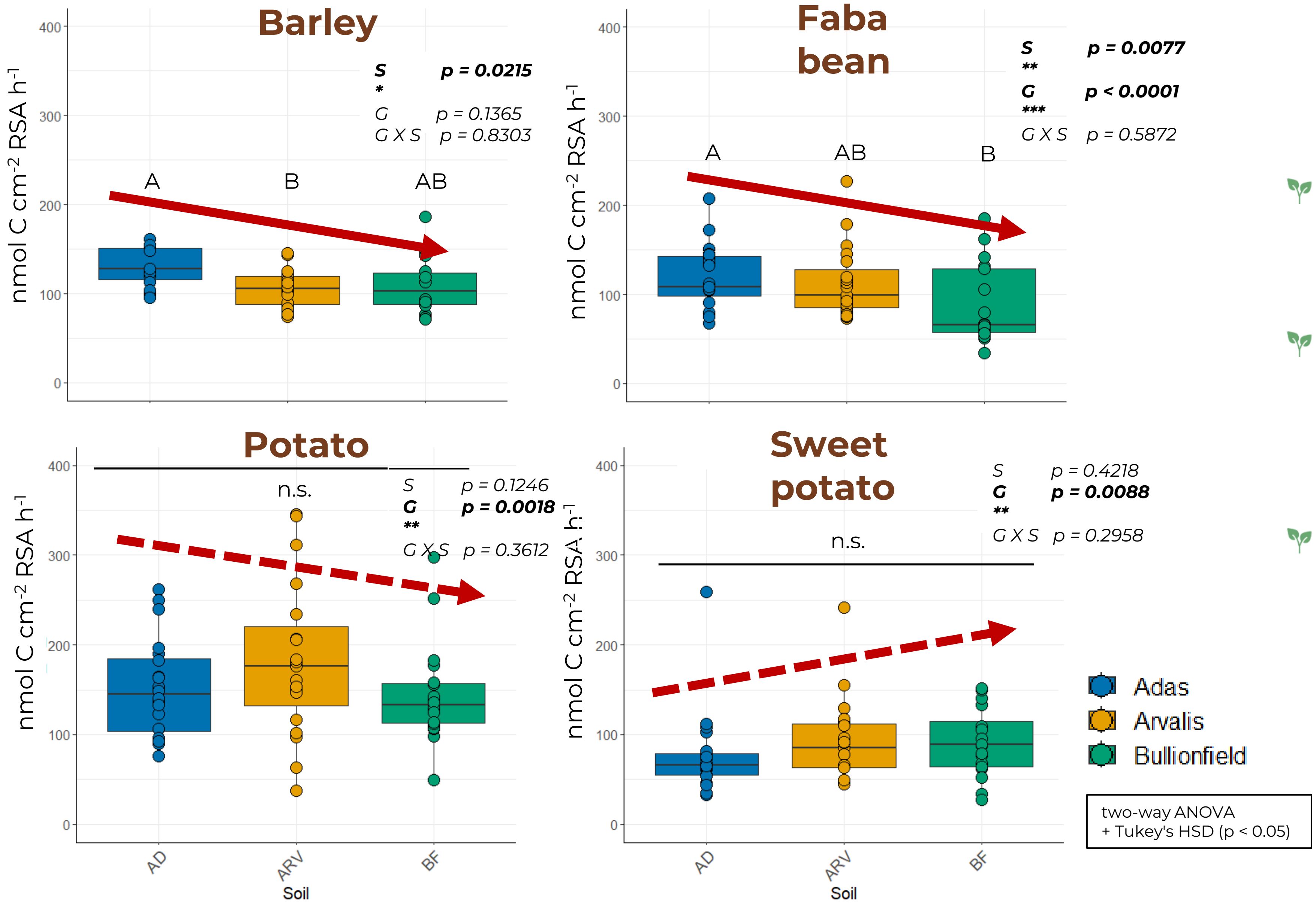
Effect of soil on C root exudation rates (nmol C cm^{-2} RSA h^{-1})



- Genotypes averaged across all soils
- Lower C exudation rates in sandy soil (with higher root biomass production)
- Potato > Faba ≥ Barley > Sweet P. but overall same order of magnitude

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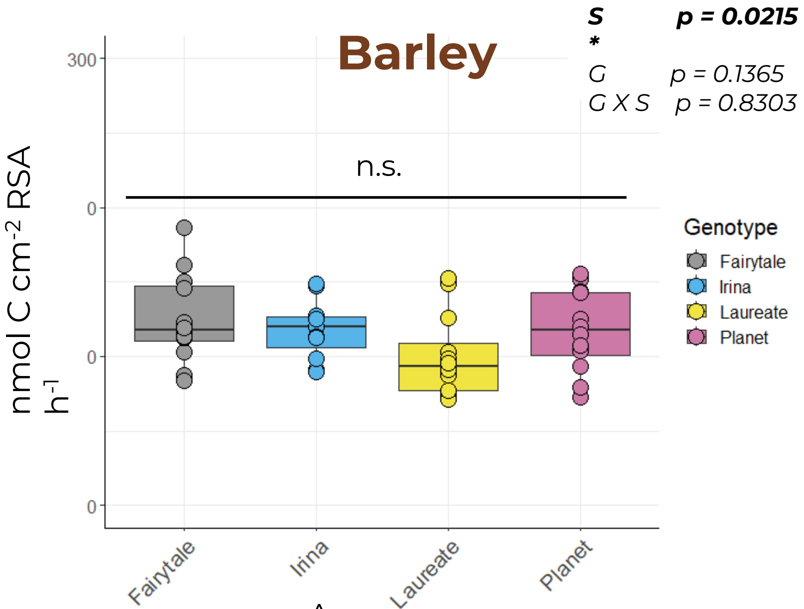
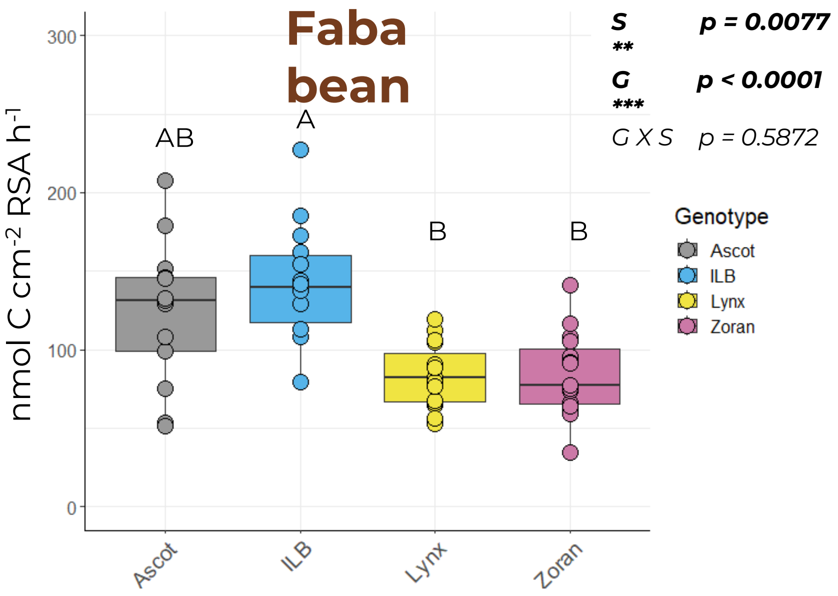
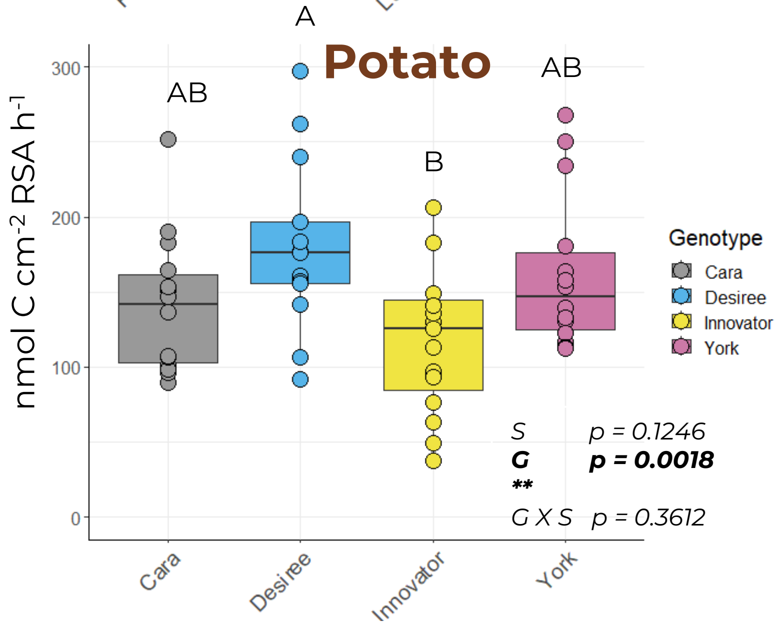
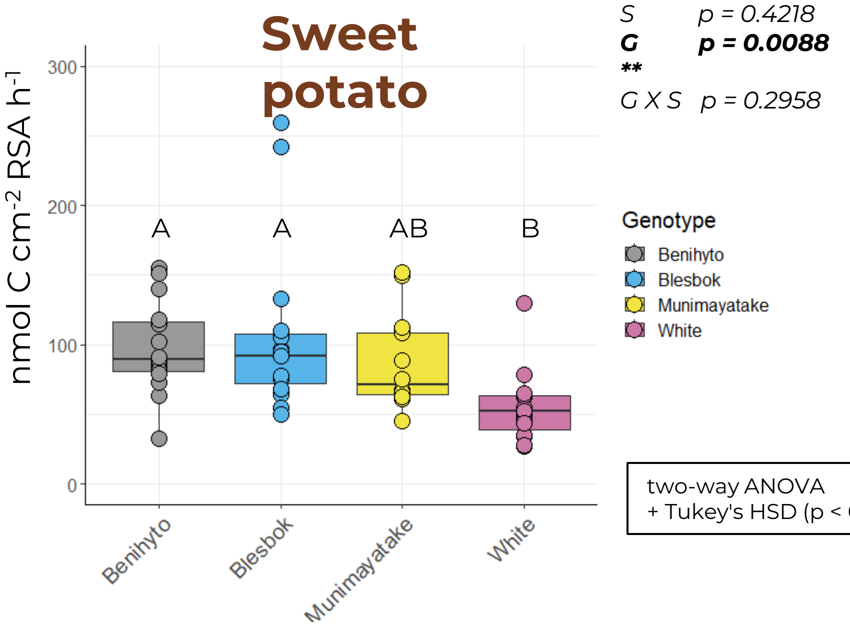
Effect of genotype on C root exudation rates (nmol C cm^{-2} RSA h^{-1})









two-way ANOVA
+ Tukey's HSD ($p < 0.05$)

- Soils averaged across genotypes
- **Genotype effect:** Faba bean, potato, sweet potato
- Barley no genotype effect

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Conclusion and perspectives

Preliminary results : definition of plasticity index and development of methodology to address rhizosphere traits

Next steps:

Extended experiments (more genotypes and environments)

All rhizosphere traits addressed with the plasticity concept:

- Architectural
- Exudate
- Associated microbiome activities
- Related root transcriptomic

Integration in the genetic toolbox of the project

Thank you for your attention

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Root phenotyping and genetic improvement for rotational crops resilient to environmental change

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