Root2Res
Root phenotyping and genetic improvement for rotational crops resilient to environmental change

Practice Abstract

How to study root plasticity in response to water availability under controlled conditions

Problem

To study the plasticity of root traits related to exploration and exploitation of soil environments, a range of methods need to be applied simultaneously. Trade-offs between sample size and resolution for visualisation as well as sample size for metabolome and transcriptome analysis and potential compromise of the quantification of root system size and diameters, need to be considered.

Solution

An 'in-depth traits assessment and understanding of plasticity' combines (1) X-ray computed tomography (CT) for the visualisation and characterisation of root system architecture (RSA) in 3D during growth (figure 1); (2) WinRHIZO-analysis for quantification of root system size and diameters; (3) root gene expression analysis (Transcriptomics) for adaption of the plants to actual growth conditions, and (4) root metabolome analysis (Metabolomics) for information on the history of growth conditions.

Benefits

By combining the above mentioned methods it is possible to study the plasticity of root systems in response to environmental conditions like differences in soil texture and chemical properties, water availability and nutrient supply.

Practical recommendations

Plant growth columns

1. Use acrylic glass tubes with a height of 25 cm, that are sealed at the bottom with a water-permeable nylon mesh (30 µm mesh size).
2. For plants with larger root diameters, e.g., faba bean, use 7 cm diameter; for plants with thinner root diameters, e.g., barley, use 5 cm diameter.
3. Fill the columns with homogenised, sieved soil up to a height of 23 cm and cover the soil with coarse gravel to minimise evaporation.
4. Place each column in a tray, which allows irrigation via capillary rise in addition to watering from the top.

Applicability box

Theme: plasticity, root system architecture, experimental systems,

Equipment: X-ray computed tomography (CT), WinRHIZO-analysis, acrylic glass tubes, climate chamber

Best in: Mineral soils

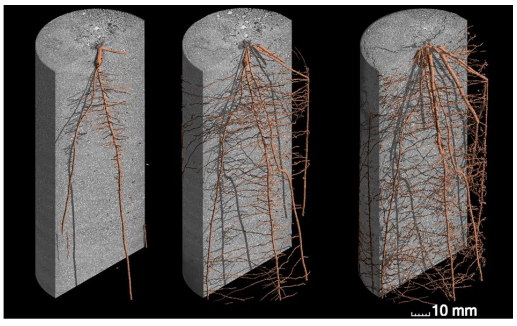

Figure 1: A maize root system in sandy soil is shown after 7, 14 and 21 days of growth in columns with a diameter of 7 cm. The spatial resolution is 45 µm. The tomograms were segmented with RootineV2 (Phalempin et al. 2021) and visualised with VGStudio Max. Figure created by Sebastian Blaser.

5. Wrap soil-filled columns with aluminium foil to minimise algal growth and root light exposure.

Climate chamber and plant growth period

- The plant growth takes place in a walk-in climate chamber, offering sufficient space (12 m^2) for the placement of numerous columns at the same time. For typical settings see table 1.

Repeated X-ray computed tomography

- To minimise disturbance of photosynthesis, perform CT imaging during the night phase in the climate chamber. For the choice of X-ray CT settings please refer to Lippold et al.

Avoid potential impacts of X-ray CT on microbiome composition and the transcriptome

- Take the samples for microbiome and transcriptome analysis more than 24 hours after the last CT scan.

- To collect roots for transcriptomic and metabolomic analysis, clean fresh roots with **dry** paper (no water should be used) and weighed. Collect fine roots using clean tweezers and a razor blade. At least 5 biological replicates should be collected for each tested condition. Root material per replicate (approximately 100 mg for transcriptomics and 100 mg for metabolomics) should be placed in 2-ml tubes, promptly frozen by using liquid nitrogen, and stored at -80°C.

Table 1: Overview of the main growth conditions for the core plant species barley and faba bean

Growth condition	Barley	Faba bean
Column diameter	5 cm	7 cm
Available soil volume (height =23 cm)	452 cm^3	885 cm^3
Day / night duration	12 h/12 h	
Temperature ranges (day / night)	19-22 °C/16-18 °C	
Photosynthetic active radiation	350 µmol m^{-2} s^{-1}	
Relative air humidity	65 %	
Growth period	3 weeks	
Optimum water treatment	*pF 2.5 with frequent re-watering	
Water logging treatment	*pF 2.5 + 10 Vol.-% water content	
Drought treatment	*pF 2.5 initially; without re-watering	

* pF = water potential (the negative decadic logarithm of the soil water tension in hectopascals). For the chosen soils, a water retention curve was determined by an evaporation experiment with HYPROP ®. Optimum water supply will be defined at pF 2.5, thus in both substrates the content of plant available water is about 18 Vol.-%.

Further information

- Lippold, Eva, et al. "Does the lack of root hairs alter root system architecture of Zea mays?." Plant and Soil 467 (2021): 267-286.
- Vetterlein, Doris, et al. "Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—laboratory and field scale." Journal of Plant Nutrition and Soil Science 184.1 (2021): 35-50.
- Phalempin M, Lippold E, Vetterlein D, Schluter S (2021) An improved method for the segmentation of roots from X-ray computed tomography 3D images: Rootine v.2. Plant methods 17: 39. doi: 10.1186/s13007-021-00735-4
- Vetterlein, Doris, et al. "Root2Res Deliverable 5.1: Development of a statistical evaluation scheme for assessing plasticity data and development of experimental systems for studying root plasticity". Available at https://root2res.eu/home/knowledge-center/

About this practice abstract and Root2Resilience

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Root2Resilience: The project is running from September 2022 to August 2027. The overall goal of Root2Resilience – Root phenotyping and genetic improvement for rotational crops resilient to environmental change – is too develop root phenotyping, genetic and modelling tools and use them to define and test innovative genotype ideotypes able to enhance the tolerance to abiotic stress and carbon sequestration in soils

Project website: root2res.eu

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