Root2Res

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

Deliverable 2.2

Workshops on root phenotyping tools

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Deliverable D2.2 reports on the various workshops organised as part of the project to train Root2Res researchers and stakeholders (end-users) in the use of the Root2Res phenotyping toolboxes.

Deliverable Number	**Work Package / Task**
D2.2	WP2 / T2.1
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ARVALIS	Katia Beauchêne, Jean-Pierre Cohan, Pierre Rochepeau (ARVALIS)
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Type of deliverable	R	Document, report (excluding periodic and final reports)	x
	DEM	Demonstrator, pilot, prototype, plan designs	
	DEC	Websites, patents filing, press & media actions, videos, etc.	
	DATA	Data sets, microdata, etc.	
	OTHER	Software, technical diagram, algorithms, models, etc.	

Dissemination level	PU	Public, fully open, e.g. web (Deliverables flagged as public will be automatically published in CORDIS project's page))	x
	SEN	Sensitive, limited under the conditions of the Grant Agreement	

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1. Introduction

Root2Res has the ambition to deliver knowledge, tools and activities, that are relevant to the diversity of agroclimatic environments encountered in Europe, with the aim of moving field and controlled environment root phenotyping towards greater resolution, reproducibility, and accuracy. This started with the development of effective root and rhizosphere phenotyping tools for field environments that can be applied to various populations of genotypes in multiple environments (D2.1 ToolBox Architectural Traits and D2.3 Phenotyping Toolbox Rhizosphere).

In order to facilitate this knowledge transfer with the project's key stakeholders and eventually ease the implementation of innovative phenotyping tools, both for field and controlled conditions, a series of workshops have been set up in each of the three agroclimatic zones studied in the project:

- The first "field" workshop was organized by ARVALIS on June 29th 2023 in Northern France (ACZ1).
- A second "controlled conditions" workshop was organized by FZJ on 5th & 6th February 2024 in Germany (ACZ2).
- Finally, a third "field" workshop was organized by ICARDA on 28th February 2024 in Morocco (ACZ3).

2. Workshop in agroclimatic zone 1

2.1. Attendees

The objective of the French workshop was to raise stakeholders' awareness of the challenges and existing methods for characterizing root systems, and more generally on the Root2Res phenotyping toolbox.

An initial stakeholder mapping exercise was carried out to target the most relevant stakeholders to invite. ARVALIS also took advantage of hosting both the international EAPR Post Harvest Section meeting and the French national potato day (Les rendez-vous techniques Pomme de terre de Villers-Saint-Christophe) at its research station of Villers-Saint-Christophe (Northern France), for a specific communication to the French actors of the potato industry.

Ultimately, through targeted interactions and promotion using a promotional leaflet (Figure 1), 32 stakeholders attended the first part of the workshop (conference presenting the project and stakes around root systems measurements for the adaptation of cropping systems to climate change) and 20 participated at the on-field demonstration of root phenotyping tools.

Figure 1: Promotional leaflet sent to targeted stakeholders for the ACZ1 root phenotyping workshop.

Different stakeholder groups were represented at the event, which corresponding to the project targets (Table 1).

Table 1: Profiles of stakeholders, who attended the ACZ1 root phenotyping workshop.

Stakeholder profile	Number
Breeders	4
Researchers/academics	8
Growers	3
Economic operators	16
Advisors	2
Sectors representatives	6
Media	1

2.2. Agenda

The agenda of the ACZ1 root phenotyping workshop is summarized in Table 2 below.

Table 2: Agenda of the ACZ1 root phenotyping workshop that took place on June 29th in Villers-Saint-Christophe, France.

TIME	AGENDA ITEM
09.50 – 10.00	Reception of attendees and welcome
10.00 – 10.15	Indoor Root2Res project presentation
10.15 – 11.00	Workshop on root phenotyping methods
14.00 – 15.00	Visit of field technical areas related to root characterization and varietal selection

2.3. Summary of exchanges

As an introduction to the workshop on root phenotyping methods, project coordinator Jean-Pierre Cohan presented the European Root2Res project and its objectives.

During the workshop, Katia Beauchêne, phenotyping expert at ARVALIS and WP2 leader then presented measuring tools adapted to controlled conditions and tools that can be used in the field to study root system characteristics such as length density, diameter, root biomass and interactions with the rhizosphere. Participants shared the need for envirotyping in studies such as this, to be able to truly characterize each trial sites characteristics and therefore accurately attribute the effects of the abiotic stresses on the root systems and cultivars' performances.

After the presentation, operational questions were raised by participants about the root phenotyping methods, which led to general discussion between them and ARVALIS experts. These interactions are summarized in Table 3 below.

Table 3. Summary of the Q&A.

N°	Questions and answers
1	**Q**: How to overcome the size limits of the root profile grid when 20% of the roots are below it? **A**: When we observe that there is still a lot of roots below the grid, we measure another grid below... We must define what is the percentage of root needed to define the root maximum depth (10% vs 1% ?).
2	**Q**: How do you distinguish the environmental effect from the root effect? **A**: Measurements are carried out in different environments that are characterized in detail, particularly regarding the soil characteristics and soil microbiome. Envirotyping also enables characterization of environmental conditions of the trials, hence allowing to consider the interaction between genotype and environment conditions to better analyze the results.
3	**Q**: Can you observe the brakes on root development linked to factors such as compaction and the impact of water resources?

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N°	Questions and answers
	A: Yes, with a soil pit you can also observe soil compaction, with minirhizotron it is more difficult
4	**Q**: How did you choose the varieties used for this study? **A**: The varieties chosen for the study were selected to represent a wide range of variability and to include varieties be able to be grown at several sites in Europe. We chose varieties for which we already know the genetic material, especially the above-ground characteristics.
5	**Q**: Have you already observed any phenotypic differences in the roots of the different varieties? Is it possible to discriminate between them? **A**: The first tests are currently purely methodological to improve the robustness of research methods, followed by tests on panels of varieties and the development of new tools based on the root traits highlighted, including the deployment of aerial methods. But we can already observe differences between crops and between genotypes.
6	**Q**: Are there any plans to develop markers? **A**: Yes, the phenotyping toolbox is also associated with two other toolboxes for modeling and genetics, including molecular markers.

Following this discussion, several demonstrations of the use of Minirhizotrons, soil pit and shovelomics were carried out in the technical area, with visitors from a wide range of backgrounds. These tool presentations led to numerous exchanges with potato experts on the challenges of root phenotyping for this crop (Figure 2).

Figure 2: Field demonstration of root phenotyping tools.

The shovelomics approach consists of measuring traits on 0 to 20 cm depth roots on a plant sampled with a spade. This method generally has a similar sampling approach for all species but the traits that were measured can be different due to the root architectural differences between cereals, potatoes, and legumes.

The soil pit method consists of digging a pit to facilitate the quantification of the presence of roots on a vertical face of the soil. The aim is to evaluate the distribution of roots and extent of soil volume influenced by roots, to determine the relationship between soil structure and rooting, and to determine a crop's capacity to adapt to the environment (water, soil mineral nitrogen). This method is usable on all crops, from flowering onwards, to assess definitive rooting.

Minirhizotron methods require the positioning of transparent tubes in the soil at a 45° angle which allows the introduction of a rotative scanner that acquires images at different depths. A set of algorithms will then allow first the detection of the percentage of root intersections per cm of soil depth by segmentation based on a deep learning approach and secondly the extraction of physical measures like root length density per area (mm of roots per cm^2 of soil) and the average root diameter per unit of soil and per depth.

The participants expressed great interest in phenotyping tools and the related scientific work initiated in Root2Res. It emerged that it will be important to be able to phenotype root systems at high throughput in order to meet breeders' economic and time constraints, as well as to provide resilient varieties to farmers as quickly as possible.

3. Workshop in agroclimatic zone 2

The root phenotyping and image analysis workshop aimed to introduce researchers and project members to innovative methods and imagining techniques for characterizing root systems and their interactions in controlled environment conditions and demonstrate some of the innovations in the Root2Res root phenotyping toolbox.

A promotional leaflet was distributed to all members of the Root2Res project, inviting them to register and attend the workshop (Figure 3). In total, 94 participants registered and participated (74 in person, 20 online), including leading experts, researchers, and new members of the Root2Res project, stakeholders including representatives of breeding companies (KWS) and agricultural advice and service providers (Eurofins) as well as participants from related projects such as RootED, WishRoots, and BarleyMicroBreed.

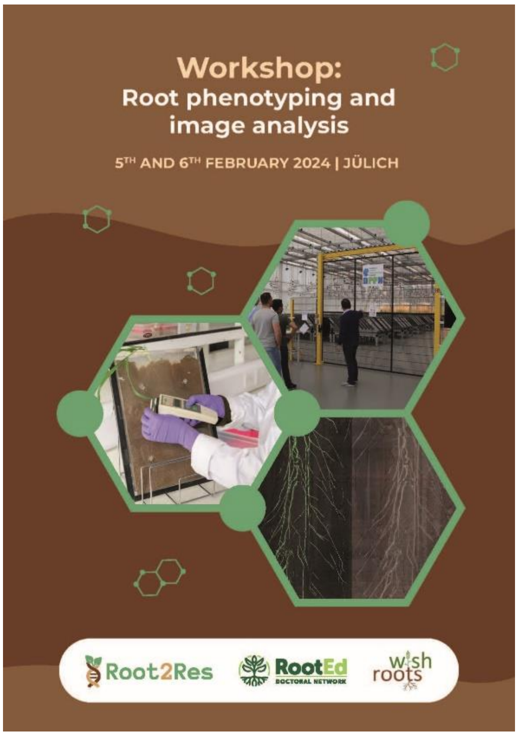

Figure 3: Promotional leaflet sent and attendance photograph for the ACZ2 root phenotyping workshop that took place at the Forschungszentrum phenotyping facilities in Jülich, Germany.

3.1. Agenda

The agenda for the Root phenotyping and image analysis workshop is outlined in Table 4.

Table 4: Agenda of the ACZ2 root phenotyping workshop held on February 5-6th 2024 in Jülich, Germany.

TIME	AGENDA ITEM
5th February 2024	
13.00 – 13.15	Introduction (Project Leads George, Tracy, Hernandez- Soriano)
13.15 – 13.55	Soil-free root phenotyping (Xavier Draye)
13.55 – 14.00	Video demo filling pots with soil (Doris Vetterlein)
14.00 – 14.25	Deciphering the interactions between plant genotype and nutrient availability (Rafaella Balestrini)
14.25 – 14.40	Access to phenotyping facilities across Europe (Roland Pieruschka)
15.00 – 16.00	Tour FZJ phenotyping facilities (Kerstin Nagel, Henning Lenz & Johannes Postma)
16.00 – 16.40	Imaging roots (Michael Pound)
16.40 – 17.00	Phenotyping roots with CT (Doris Vetterlein)

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17.00 – 17.15	Phenotyping roots with MRI & PET (Daniel Pflugfelder)
17.15 – 17.20	Arvalis minirhizotron video
17.20 – 17.40	From controlled environments to the field (Silvio Salvi)
17.40 – 17.45	Video demo on shovelomics/field phenotyping (Silvio Salvi)
18.00 – 21.00	Networking dinner
6th February 2024	
09.00 – 09.45	How easy is it to grow plants for experimental purposes? (Hendrik Poorter)
09.45 – 10.30	Sampling rhizosphere soils for microbes (Tim George)
11.00 – 11.45	Demo Block 1 • Gnotobiotic systems (e.g. Ecofabs) (Borjana Arsova) • Root washing and imaging (Nicolai Jablowski) • GrowScreenAgar 2 & Rhizovision (Hamid Rouina)
11.45 – 12.30	Demo Block 2 • Rhizosphere sampling (Henning Schwalm, Tim George) • Winrhizo & Smartroot (Helena Bochmann & Xavier Draye) • 3D phenotyping in soil (Daniel Pflugfelder & Dusschoten)

3.2. Summary of exchanges

The workshop started with an introduction to the Root2Res project, followed by talks from leading experts on the theoretical aspects of root phenotyping tools. Attendees were introduced to techniques for examining root systems in natural soil conditions or soil-free settings like hydroponics and aeroponics. Advanced imaging techniques were introduced (e.g., CT scans, MRI, PET, fluorescence, and optical imaging) to visualize and analyze the architecture of plant roots, including their length, branching patterns, diameter, and root hairs and their role in water and nutrient uptake as well as resilience to stress. Discussions were held on the limitations of each technique, and guidelines were provided for their appropriate use (i.e., repeated exposure of plants to CT imaging will cause damage to plant tissues due to ionizing radiation; while MRI imaging offers advantages such as tracking water flow, its resolution is insufficient for visualizing finer details like root hairs).

The workshop emphasized the importance of moving from a controlled laboratory environment to field conditions, addressing the challenges of fieldwork, and introducing techniques like shovelomics for field phenotyping. Experts shared insights on common pitfalls in designing plant experiments, emphasizing the impact of factors like light intensity and randomization on plant growth in laboratory conditions versus the field. This led to discussions on designing experiments that mimic natural conditions as closely as possible to improve plant growth studies.

A protocol for sampling both rhizosphere soil and root exudates from the same plant, developed within the Root2Res project, was described. Practical and detailed videos were shared during the workshop, demonstrating techniques for filling pots with soil to ensure uniform distribution and sampling soil for microbiome analysis. The event concluded with hands-on demonstration sessions, where experts showcased various root phenotyping techniques, allowing participants to learn how these methods could be applied in their experiments (Figure 4).

Figure 4: Controlled laboratory environment demonstration of root phenotyping tools.

Stakeholders expressed the opinion that the content of the workshop was very useful for their needs, highlighting tips and pitfalls for working with the various systems. All mentioned the importance of having effective root and rhizosphere phenotyping tools.

The workshop served as a platform for knowledge exchange, fostering collaboration and sharing best practices in root system analysis and phenotyping across the different agroclimatic zones.

4. Workshop in agroclimatic zone 3

4.1. Attendees

The objective of the Moroccan workshop was to raise ACZ3 stakeholders' awareness of the challenges and existing methods for characterizing root systems in arid areas and demonstrate some of the innovations in the Root2Res root phenotyping toolbox. It was organized the day after the sister project BarleyMicroBreed Annual Meeting to maximize the number of participants.

In addition, a specific promotion of the ACZ3 workshop had been anticipated, using a promotional leaflet (Figure 1) and targeted invitation emails. All in all, 42 stakeholders from various countries (Austria, Denmark, Sweden, Italy, Morocco, Senegal and Ethiopia) attended the workshop, including all the targeted profiles: researchers, breeders, farmers, professors, master students and PhD candidates.

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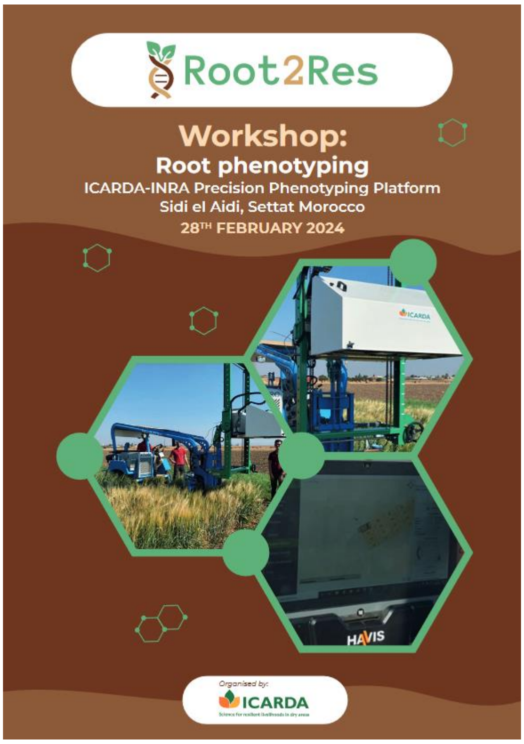

Figure 5: Promotional leaflet sent and attendance photograph for the ACZ3 root phenotyping workshop that took place at the Sidi el Aidi Station of ICARDA-INRA and photographs of a few attendants.

4.2. Agenda

The agenda for the Root phenotyping and image analysis workshop is outlined in Table 4.

Table 5: Agenda of the ACZ3 root phenotyping workshop held on February 28th 2024 in Sidi el Aidi, Settat, Morocco

TIME	AGENDA ITEM
28th February 2024	
09.00 – 09.15	Introduction to Root2Res and BarleyMicroBreed projects and to the workshop (Andrea Visioni and Miguel Sanchez Garcia and ICARDA team)
09.15 – 10.45	Phenobuggy and Physiotron practical demonstration and discussion with R2R and BMB project members (Andrea Visioni)
11.30 – 13.00	Soil Coring sampling and data collection with WinRhizo (Anna Backhaus, Andrea Visioni and ICARDA Team)
14.00 – 16.00	Shovelomics: sampling and data collection with shovelomic board and WinRhizo and other online free resources
16.00 – 17.00	Introduction to data analysis, machine learning and phenomic selection (Zineb Dagdad and Safaa Ouaid)

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4.3. Summary of exchanges

The workshop started with an introduction to the Root2Res project, followed by a demonstration of the ICARDA-INRA Heat and Drought Precision Phenotyping Platform (Figure 6). The precision phenotyping platform, created in collaboration with the National Institute for Agricultural Research of Morocco (INRA) and CIMMYT, hosts a rainout shelter fully automated lysimeter (PhysioTron) equipped with an HTP system designed for drought and heat stress tolerance studies. Data collected from those and other ICARDA strategic testing locations across its mandate region are combined and used to provide elite germplasm to NARES partners. This HTP system measurement is also used in Root2Res at ARVALIS' stations of Ouzouer-Le-Marché (ACZ1-2-3) and in Gréoux-les-Bains (ACZ3).

Figure 6: a) Physiotron facilities overview, b) Details of the fully automated system, c) Details of the HTP system measurement head, d) Overview of the Sidi el Aidi Station

Partners also had the opportunity to see the Phenobuggy: a tractor-mounted, GPS-assisted multi-sensor head.
Discussions were held on the crop traits that these devices are able to capture and their importance in the breeding schemes. The device allows for rapid and accurate measuring of phenotypic traits, such as green fraction, senescence, vegetation indices, leaf area index, biomass, spike number, and plant height.
Discussions also emerged about the necessity to develop proxies approaches to link above-ground measurements to root traits.

Following sessions were about soil coring, root seminal angle measurement with the clear pot method and as well as shovelomics sampling and data collection with WinRhizo. WinRhizo is an image analysis system specifically designed for root measurement in different forms. It can operate morphology (length, area, volume...), architecture, topology and colour analyses. It is made of a computer software and image acquisition components that can be combined to meet different needs and budgets.
Discussions focused on the importance of standardizing the characteristics of root systems for image analysis. The dependency of errors on the response variable of

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interest can influence the effect size and increase the probability of errors. Validation of methods should be conducted for each dataset. Finally, the importance of how root system morphology affects crop adaptation to different soil types was also discussed taking as example ICARDA and INRA experiences at different experimental stations in Morocco.

The last part of the workshop focused on data analysis, machine learning and phenomic selection. Breeders emphasized the need for approaches enabling evaluation as precisely as possible of the genetic variability of quantitative traits as they are crucial in the context of breeding. It requires accurate, rapid and cost-effective evaluation tools. For complex traits like yield, this can be assisted by genomic selection, which is based on estimating breeding values with genome-wide marker data. Alternatively, phenomic prediction, based on multimodal machine learning model which, similar to its genomic counterpart, aims to predict the performance of untested individuals but combining genomic, phenomic and environmental data. Participants agreed upon the fact that this prediction approach is a compatible way to increase genetic gain in plant breeding.

Through collaboration and the sharing of best practices in root system analysis and phenotyping, the workshop acted as a privileged forum for the exchange of information relevant for ACZ3 agropedoclimatic conditions and specificities.

5. Conclusion

This series of workshops in each of the 3 agroclimatic zones received positive feedback from participants and Root2Res organizing partners in particular for 3 main reasons: i) there was a need from breeders, agronomists and farmers to be given a privileged place for exchange with the scientific community about the operational root phenotyping methods and potential insertion in breeding schemes, ii) it raised the importance to share understanding of the limitation of current root phenotyping methods and pathways to work on in the future to be able to make them more high throughput and scalable from trial conditions to actual breeding conditions, iii) provide a live-demonstration of protocols to set up, ways to implement them and how to interpret imaged-data collected.

In accordance with the stakeholder engagement and interaction strategy included in D7.2 Dissemination and Exploitation Plans (including the communication handbook), all attendees have been integrated within the Root2Res stakeholder list and will be kept informed of the update of the phenotyping toolbox that will be finessed within the coming year with our results from the project's experiments on barley, durum wheat, potato, sweet potato, faba bean, pea, and lentil.