Root2Res

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

Deliverable 2.1

Phenotyping toolbox

-

Architectural traits

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Deliverable D2.1 comprises a list of phenotyping tools suitable for quantifying root architectural traits applicable for field trials and CE experiments. This list will be compiled and associated to practice abstracts. It will be one of the basement of the 'phenotyping toolbox' (T2.1).

Deliverable Number	Work Package / Task
D 2.1	WP2 T1
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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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INDEX

FIGURES

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TABLES

1. Introduction

The overall ambition of Root2Res is to identify and develop more resilient crop cultivars capable to mitigate the destabilizing impacts of climate change. Root and rhizosphere traits play a major role in climate resilience as they determine water and nutrient uptake contributing to an improved tolerance against abiotic stresses (De Vries et al., 2020; Ober et al., 2021). Key to the Root2Res approach is therefore the provision of tools for phenotyping root and rhizosphere traits. We will present here a toolbox for phenotyping root architectural traits in the field and in controlled environments. This toolbox will be finessed within the coming year with our results from Root2Res experiments on barley, durum wheat, potato, sweet potato, faba bean, pea, and lentil.

2. Phenotyping architectural traits in field environment

In the field environment, there are a number of already developed methods which allow the measurement of architectural traits. Within the Root2Res consortium, we worked together to develop these methods for the species of interest and shared standard operating procedure (SOP) for each method of phenotyping already used by at least one partner.

2.1. Root architectural traits using “shovelomics”

The “Shovelomics” approach consists of measuring traits on 0 to 20 cm depth roots on a plant sampled with a spade (Figure 1). For the Root2Res project, the “Shovelomics” method is based on the ADAS standard operating procedure (SOP) used for cereals and legumes. This SOP was adapted for the requirements of each species. The adapted methods generally had a similar sampling approach (Table 1) but the traits that were measured were different due to the root architecture differences between cereals, potatoes, and legumes (Table 2). For example, literature precedents and excavation of plants show that some traits are relevant to one species and not another like roots associated with stolons for potato compared to the tap root system of beans.

Figure 1: Spade to shovel sample on barley (left) and on a potato (middle) field trial. Maize Shovelomics Scoreboard (right) used to record barley root system angle (to access the scoreboard please use this link: https://plantscience.psu.edu/research/labs/roots/).

Table 1. General description of the sampling method.

Depth	Sampling	Materials
0 to 25 cm (spade size)	4-5 plants per plot (could be more if you want something more precise)	Measurements collected by hand, no software required, just spades, bags, and scoreboard.

Time*	
Cereals: (1 sample = 5 plants)	**Potato**: (1 sample = 5 plants)
• 7.5 min to sample 5 plants per plot in the field. • 15 min to wash 5 plants in the lab. • 45 min to measure the shovelomics traits. **TOTAL for 5 plants = 1h05**	• 50 min to sample 5 plants in the field. • 45 min to wash 5 plants in the lab. • 3 h 15 min to measure the shovelomics traits and to scan the samples with Winrhizo **TOTAL for 5 plants = 5 h**
Legumes: to be determined	

* Timings here are an estimation. These will be refined when the SOPs have been tested in the field across the ROOT2RES network.

Table 2: List traits evaluated with shovelomics for each crop.

Cereals	
• Tiller (number) • Root width at max (mm) • Root width 90° (mm) • Depth to maximum width (mm) • Angle of root 1 (°) • Angle of root 2 (°) • Nodal root length (L) (mm) • Nodal root length (R) (mm)	• Nodal root branching density (scale 1-4) • Nodal root number (Tiller 1) • Seminal Root number (number) • Seminal root branching density (scale 1-4) • Dry weight roots (g)
Faba Beans	
• Nodulation No. (scale 1-9) • Nodule size (mm) • Active nodules (number) • Nodulation score (scale 1-8) • Stem width (mm) • Tap root diameter (mm) • Root width at max (mm) • Root width 90° (mm) • Depth to maximum width (mm) • Maximum root depth retrieved (mm) • Angle of root 1 (°) • Angle of root 2 (°) • Epicotyl root length (L) (mm)	• Epicotyl root branching density (scale 1-4) • Primary root length (mm) • No. Primary root laterals (number) • Primary root branching density (scale 1-9) • Lateral root branching density (scale 1-4) • Fresh Weight above ground biomass (g) • Dry weight above ground biomass (g) • Filter Dry wt (g) • Dry wt filter+roots (g) • Dry weight roots (g)

• Epicotyl root length (R) (mm) • Epicotyl root number (number)
Potato
• Tuber weight (g) • Stolon weight (g) • Stem weight (g) • Leaf weight (g) • 1st leaf weight (g) • Total leaf weight (g) • Extra tuber weight (g) • S/S Junction Root weight (g) • Stolon root weight (g) • Basal root weight (g) • Extra root weight (g) • Total root weight (g) • S/S Junction Root length (cm) • Stolon root length (cm) • Basal root length (cm) • Extra root length (cm) • Av s/s junction root diam (mm) • Av Stolon root diam (mm) • Av Basal root diam (mm) • Av Extra root diam (mm) • S/S Junction root SA (cm) • Stolon root SA (cm) • Basal root SA (cm) • Extra root SA (cm) • No plantlets (number) • No stolons (number) • No leaves (number) • No of stolon roots (number) • No of stolon node roots (number) • No of nodes no (number) • Secondary stolon no (number) • Stolon length (cm) • No of stolons (number) • No of leaves (number) • No of basal roots (number)

For details of the Shovelomics SOPs, see Annex 1 for faba beans; Annex 2 for barley, and Annex 3 for potato.

Relevant publications

Trachsel, S., et al. (2010). Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil*; doi 10.1007/s11104-010-0623-8.

York, L. M. et al. (2018). Wheat shovelomics I: A field phenotyping approach for characterising the structure and function of root systems in tillering species. *BioRxiv*; doi 10.1101/280875.

Slack, S., et al. (2018). Wheat shovelomics II: Revealing relationships between root crown traits and crop growth. *BioRxiv*; doi 10.1101/280917.

Fradgley, N., et al. (2020). Effects of breeding history and crop management on the root architecture of wheat. *Plant and Soil*; doi 10.1007/s11104-020-04585-2.

2.2. Root architectural traits derived from “soil coring”

The “Soil coring” method consists of sampling a core of soil of about 90 cm depth and extracting the roots from predetermined depths (every 20 cm). The roots collected are weighed for biomass but can also be analysed by WinRhizo for root length, root surface area and root diameter (Figure 2). For the Root2Res project, the “Soil coring” method is based on the ADAS standard operating procedure (Table 3 and Table 4).

Figure 2: 1-Soil corer in barley field trial. 2-Washing machine. 3-Manual washing. 4-Clean root samples. 5-Root scanning. 6-Root analysis by WinRhizo.

Table 3. General description of the sampling method.

Depth	Sampling	Materials
0 to 90/100 cm (core size)	volume of about 500/1000 cm^3 of soil each 20 cm depth	Measurements collected by a coring machine (about 36K€), tubes, freezer, a washing system (about 500€), WinRhizo scanner and software (about 7K€), a balance 0.001 g (about 2K€)
Time*		
3 steps for this method: (1 sample = 1 tube) • 60 min / plot to sample (3 area per plot) • 4h25 / plot to wash soil samples and extract roots • TBC to scan each sample with WinRhizo • TBC to measure the biomass		

* Timings here are an estimation. These will be refined when the SOPs have been tested in the field across the ROOT2RES network.

Table 4: List traits evaluated with this method.

All crops	
• Root biomass (g (Dry Weight) per cm^3 of soil) • Total root Length(cm) • Root surface area(cm^2)	• Root Average Diameter (mm) • Root Length Per Volume (cm/m^3) • Root Volume (cm^3)

More details, see Annex 4 "Soil Coring standard Operating procedure and Root Analysis of Field Sampled Roots".

Relevant publications

Clarke, C. K., et al. (2017). Quantifying rooting at depth in a wheat doubled haploid populations with introgression from wild emmer. Annals of Botany; doi 10.1093/aob/mcx068.

White, C. A., et al. (2015). Root length densities of UK wheat and oilseed rape crops with implications for water capture and yield. Journal of Experimental Botany; doi 10.1093/jxb/erv077.

For WinRhizo see : https://regent.qc.ca/assets/references.html#winrhizo

2.3. Dynamic of Root length density and root diameter per depth with scanning by "Minirhizotron" method

This method requires 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 (Figure 3). A set of processing algorithms allows 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 cm² of soil) and the average root diameter per unit of soil and per depth. For the Root2Res project, the "Minirhizotron" method is based on the ARVALIS standard operating procedure (Table 5 and Table 6). ARVALIS has developed approaches to extract data from the images generated (ARVALIS Software).

- The first step of processing data is to segment the photo to detect each root. For that we used a deep learning module based on 50 annotated photos from different location with various background and different species (bread wheat, durum wheat, maize and flax). The Root2Res Project will add annotated pictures of potato and barley to this library. From this segmentation, dividing by 1 cm pixel, grid information that could be comparable to a root profile data is extracted.
- The second step of processing the data is to extract physical information from each root that are segmented. First is the sum of total root length by picture (so each 15 cm depth) and second is the average diameter of the same roots. These two traits are automatically extracted from each picture, but the distribution of root diameter from each depth measured could also be extracted.

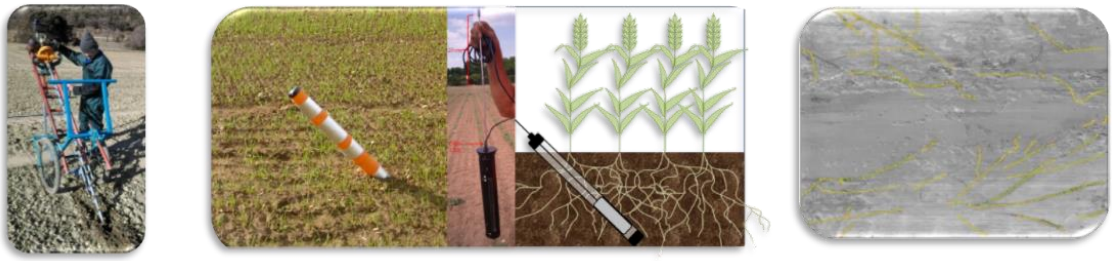

Figure 3: 1-Drilling machine to put transparent tube into the soil (45° angle) .2-tube protection, rotative scanner. 3-Picture taken with the scanner and processed with the ARVALIS segmentation.

Table 5. General description of the sampling method.

Depth	Sampling	Materials
The transparent tube is 2 m long, introducing the tube at 45° you will be able to measure between 80 and 120 cm depth. **For cereals**: the minirhizotron data are not available from the first 30 cm depth so you will have data between –30 and –100 cm depth.	one to 3 tube per plot	Scanner CI-600 by CID BIOSCIENCE (20K€), a transparent tube with sealing stopper for the bottom and a cap for the top (35€), a drilling machine (approx. 30K€)

Time
• 4 tubes/h/pers to put tubes into soil with a drilling machine, 1 tube /h / pers with a hand auger. • 15 min/tubes/pers to sample pictures on each tube (about 7 per tubes), plan no more than 30 tubes per day • Image backup and data processing (ARVALIS Software)

Table 6: List traits evaluated with this method.

All crops
• Total Root length per depth(mm) • Total Root length density (mm of root per cm² of soil view by the scanner) per depth • Average of root diameter (mm) per depth

For more details, see in Annex 5: Minirhizotron Standard Operating Procedure (installation of tubes and Root endoscopy measurements in field).

Relevant Publications

Hansson, A.C. and Andrén, O. (1987) 'Root dynamics in barley, lucerne and meadow fescue investigated with a mini-rhizotron technique', Plant and Soil, 103(1), pp. 33–38. Available at https://doi.org/10.1007/BF02370664.

Lebègue, C. et al. (2004) 'Mise en œuvre de carottages de sol et de minirhizotrons pour l'étude à long terme de la réponse des fines racines d'épicéa (Picea abies (L.) Karst.) à l'augmentation de la concentration en CO2 dans l'atmosphère et la nutrition minérale', BASE [Preprint]. Available at: https://popups.uliege.be/1780-4507/index.php?id=13964 (Accessed: 21 March 2023).

Postic, F., Beauchêne, K., Gouache, D., Doussan, C., 2019. Scanner-Based Minirhizotrons Help to Highlight Relations between Deep Roots and Yield in Various Wheat Cultivars under Combined Water and Nitrogen Deficit Conditions. Agronomy 9, 297. https://doi.org/10.3390/agronomy9060297.

2.4. Root distribution measured in a "soil pit"

For this method, a pit needs to be dug and the presence of roots on a vertical face of the soil noted (Figure 4). The aim is to evaluate the distribution 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. For the Root2Res project, the "soil pit" method is based on the ARVALIS standard operating procedure (Table 7 and Table 8).

Figure 4: Measurements in a soil pit on barley field trial.

Table 7. General description of the sampling method.

Depth	Sampling	Materials
0 to 100-120 cm (pit depth)	This depends of the size of the grid, (80 cm x 80 cm in France) • **Cereals:** 2 grids depth, one below the other = 1.28 m² on a vertical face of the soil • **Potato**: 2 grids, one next the other = 1.28 m² on an horizontal face of the soil	Backhoe to dig the pit, rigid blade knife, Grid (e.g. 80*80 cm) with 2 cm square meshes, 2 operators in the field one who measure and one who takes notes.
Time*		
3 steps for this method • 1h with 1 person: Dig the pit with a backhoe; • 15 min with 1 person Finish the hole with a shovel and prepare the observation face; • 1h with 2 people : observe rooting and record on a sheet of paper; • 1h with 1 person to close the pit.		

* Timings here are an estimation. These will be refined when the SOPs have been tested in the field across the ROOT2RES network.

Table 8: List traits evaluated with this method.

All crops
• Presence / absence of root per cm² • Percentage of root per cm of soil depth

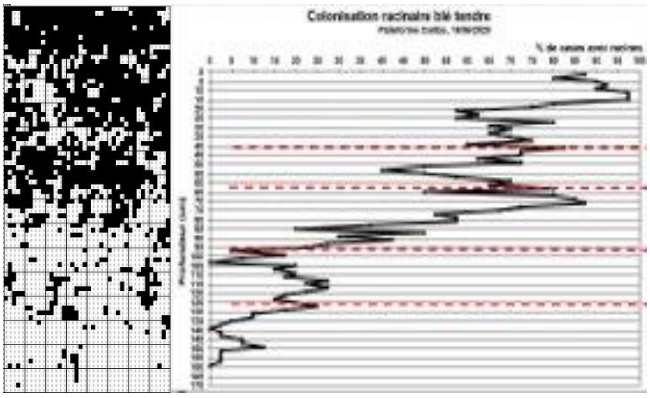

Figure 5: 1-Presence / absence of root per cm². 2-Percentage of root per cm of soil depth

For more details, see Annex 6: Soil Pit standard operating procedure: Notation of crop rooting.

Relevant publications

Zarzyńska, K., Boguszewska-Mańkowska, D. and Nosalewicz, A. (2017) 'Differences in size and architecture of the potato cultivars root system and their tolerance to drought stress', *Plant, Soil and Environment*, 63(4), pp. 159–164. Available at: https://doi.org/10.17221/4/2017-PSE.

2.5. Summary table: phenotyping toolbox for architectural traits in field environment

Method	Material (Cost)	Depth measured - Sample size	Time consuming	Traits
Shovelomics: Measuring traits on 0 to 20 cm depth roots on a plant sampled with a spade.	Spade 40€ WinRhizo (scanner + software) 7K€	0 cm to 25 cm - 5 plants per plot	**Cereals:** 1 Sample = 5 plants Sampling: 7.5 min, Cleaning: 15 min, Measure: 42 min TOTAL for 5 plants = 1h **Potato**: 1 Sample = 5 plants Sampling + Cleaning: 1h45 Measure + scanner: 3h15 TOTAL for 5 plants = 5h	**Cereals**: no. of tillers; root angle; total root length; branching density; maximum width; depth to maximum width; root/ shoot ratio **Potato**: no. plantlet; no, length and biomass stolon; no, length and biomass of basal roots; no, length and biomass of stolon roots
Soil coring: Sampling a core of soil of about 90 cm depth with an hydrocare and extract from each depth (every 20 cm) all the roots.	Hydrocare 36K€ Washing machine 500€ WinRhizo (scanner + software) 7K€	0 cm to 90 cm - 6 core samples/plot if 3 cm diameter	sample: 60 min / plot Note: 4 hours / plot TOTAL for 1 plot= 5h	total root length, root length density (cm root/cm3 of soil), root diameter average and root biomass per depth (every 20 cm)
Minirhizotron: Introduce tubes into the field, take pictures with a rotative scanner and analyse picture to extract data	Rotative scanner 22K€ Tubes 40€ Analysis software (internal ARVALIS)	40 cm to 100 cm - 0.06 m^2	Tube: once a year 15 min / tube /pers Note 15 min / tube /pers Analyse (software): 1 min all pictures TOTAL per tube per date = 20 min	total root length density (mm root/cm^2 of soil), root diameter average (µm) per depth (every 20 cm)
Soil pit: Dig a pit and note each cm square with a root using a grid	backhoe location grid counting (80 cm x 80 cm)	0 cm to 150 cm - 0.6 to 1.2 m^2	1 Sample = 80*80 cm 0.64m^2 Dig the pit: 1h Preparation + Measure: 3h TOTAL for 1 grid (1 plot) = 4h	Percentage of cm^2 with root

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3. Phenotyping root architectural traits in a controlled environment

3.1. Phenotyping root biomass in controlled environment (CE) experiments

Phenotyping of root biomass from plants grown in pots, soil columns or rhizotrons in CE environments requires destructive sampling followed by root washing. After washing off soil thoroughly from roots, shoot and root get separated and dried in an oven at 60°C for 72 hours. Dry weight of shoot and root are recorded.

The traits evaluated with this method are:

- Shoot biomass (g)
- Root biomass (g)
- Root/ shoot ratio

3.2. Phenotyping root architectural traits in pots or soil columns

Phenotyping of root architecture and root morphology from plants grown in pots or soil columns requires destructive sampling followed by root washing. Either the whole root system or a representative aliquot can be stored in 30% ethanol (ETOH) and subsequently subjected to analysis via a root scanning software, e.g. WinRhizo. For image scanning, roots are spread out in a plastic tray filled with water. The scanned root image is then analysed with a suitable root imaging software to obtain morphological traits (Figure 6).

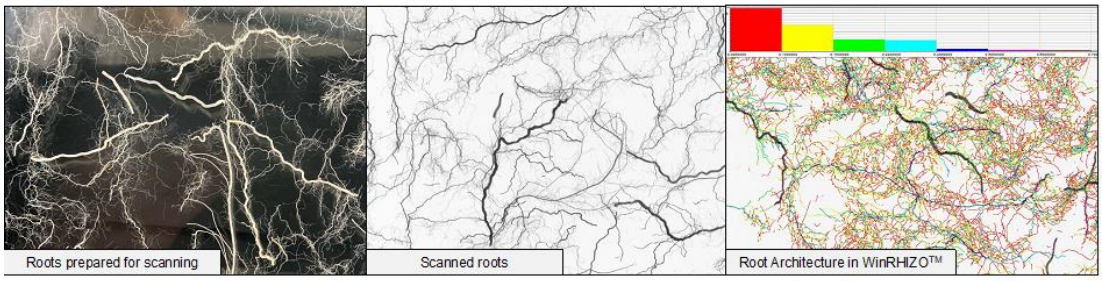

Figure 6: Example of a WinRhizo root scan

The traits evaluated with this method are:

- total root length (cm)
- total root surface area (cm^2)
- root diameter classes (no per width classes)
- Root volume (cm3)

3.3. Phenotyping root architectural traits in rhizotrons

Rhizotrons are flat growth boxes usually equipped with a transparent plate allowing the continuous monitoring of root growth performance with time. Typically, root windows are positioned at a 45°C angle forcing the root to grow along the observation window due to gravitropism. In combination with an automated scanning system combined with an image analysis software, this setup enables automated observation of the root system at the soil-observation window interface in CE with plant development.

The traits evaluated with this method are:

- root vigour
- root growth (rate)
- root branching (no)
- root angle (°)
- root depth (cm)
- convex area (cm^2)

3.4. Phenotyping root architectural traits in long tubes

In Italy (CNR), root phenotyping facility is located at ALSIA (Metaponto, Matera, Italy), and phenotyping is performed by using glass-made tubes. The glass-made tubes are cylindrical transparent containers used to study root growth under controlled conditions. The tubes have a length of 60 cm and a diameter of 10 cm (Figure 7A). This design allows the monitoring of the root system as it develops, and the transparency of the glass ensured that the entire root system is visible. The cylindrical shape, coupled with its dimensions, offers ample space for the roots to grow, ensuring that the plant is not constrained, and that root growth is as natural as possible within the limits of the tubes (Figure 7B). Tubes are filled with specific substrates (e.g., soil poor in nutrients) to both allow fine tuning modulation of nutrient content and a standardized procedure for watering the plants.

The phenotyping facility harbours a cutting-edge computer vision software designed to evaluate the architecture of plant root systems. The facility is equipped with rotating cylindrical rhizotrons, which facilitates multiple imaging perspectives of the roots. By taking multiple shots at various angles, a holistic view of the root system is ensured. Individual images captured are merged, resulting in a panorama image that offers a 360° perspective of the root layout (Figure 7C). In addition, the facility employs rotation correction techniques and advanced image transformation processes. These methods ensure that any potential artifacts introduced during the rotational imaging are effectively addressed, leading to high-fidelity root images. This procedure is carried out based on the number of images (ranging from 4 to 6) captured during the complete 360° rotation. The Singular Value Decomposition (SVD) algorithm is used to attenuate noise by focusing on the lower rank approximation of the panorama, ensuring high resolution of resulting images. Through automatic segmentation, images of roots are isolated by effectively filtering out the Laplacian of Gaussian of PCLEAN. This results in binary images where the roots are distinctly recorded (Figure 7C).

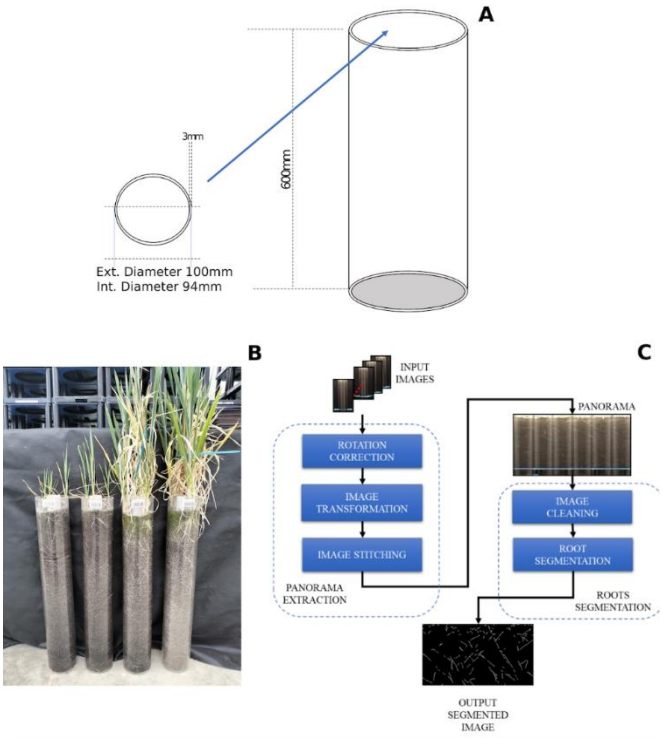

Figure 7: Tubes used in root phenotyping facility in Italy. In (A), schematic representation of the glass-made tubes; in (B), example of bread wheat plants grown in the tubes and subjected to different level of phosphorous in the substrate; in (C), flow diagram showing the steps of the panorama extraction and root segmentation performed by the software used in the facility.

The traits evaluated with this method are:

- Root growth over time (cm^2)
- Root depth (cm)
- Root total are (cm^2)
- Root biomass (after sampling) (g).

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ANNEXES

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1. Shovelomics Standard Operating Procedure for faba bean

Version 2: April 2023

Usually taken in the spring prior to stem extension but can be done at any time. This protocol is an amalgamation of the maize and bean shovelomics work at Penn State University, USA and designed for faba bean, other beans may have a different root system architecture type and may need different measurements or terminology. The protocol is based on sampling plants in an experimental field plot trial. If you have any questions or comments, please get in touch.

Authors

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Charlotte White, charlotte.white@adas.co.uk

Relevant publications

Trachsel, S., et al. (2010). Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil*; doi 10.1007/s11104-010-0623-8.

Burridge J., et al. (2016) Legume shovelomics: High-Throughput phenotyping of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* subsp, *unguiculata*) root architecture in the field. *Field Crops Research*; doi 10.1016/j.fcr.2016.04.008.

Burridge JD, Rangarajan H, Lynch JP. Comparative phenomics of annual grain legume root architecture. Crop Science, 2020;60:2574-2593

Time required

- 0.25 hours to sample 5 plants (single plot) in the field
- 2.2 hours to analyse 5 plants (single plot) in the lab

Materials and Equipment required for field sampling

- Spade
- Sampling bags
- Marker
- Trial plot map

Materials and Equipment required for lab analysis

- Pen/paper or laptop to record measurements
- Laminated 'Maize Shovelomics Board' or DIY Bean board.
- Black A3 sheet of card
- >10 litre buckets and soap to soak plants
- Camera and tripod
- Ruler
- Callipers
- Tweezers for handling and separating out roots
- WinRhizo software, flatbed scanner, and scanning trays (optional)
- Drying oven, oven trays, filter paper, and scales which measure to four decimal places to measure above/belowground biomass (optional)

Timing of measurement

Measurements can be done anytime from just prior to stem extension to flowering. It is best performed when the soil is moist and soft. For beans, excavation 35-45 days after planting (DAP) is recommended in the literature. Evaluation during the flowering period minimises differences in phenology among lines that may affect root development. Also, at flowering the mature root phenotype is evident.

For the Root2Res project we are targeting 35-45 days after planting for shovelomics assessment.

Excavation of plants

In each plot, excavate five plants from a range of sample points in the plot. The aim is to extract 5 entire and undisturbed root crowns from each plot. Depending on planting density it may be necessary to excavate more than one plant together. If removing groups of plants sample two or three groups of plants from different rows, in different areas of the plot, avoiding the outer two rows.

Excavate each plant using a spade. Select one plant, or group of plants, remove the top 20 cm (or the full length of the spade) of soil around the plant to ensure the full root crown is removed. So that there's a cylinder with 20 cm diameter around the plant.

- Insert the spade 20 cm deep on either side of each plant, with the width of the spade parallel to the row.
- Ensure approximately the same volume of soil is removed for each plant sampled.

Place removed plants into a labelled sample bag to transport back to the lab. Care should be taken to avoid disturbing the soil attached to the crown too much.

- If some of the soil comes away from the roots, it is not necessary to place it in the sample bag, as long as all the plant material is sampled from within the volume of soil removed.

Bean roots are especially fragile more care needs to be taken with these samples.

Root crown assessments (in the lab)

Root crowns from the same plot can be assessed together.

- Place the five root crowns from each plot into >10 litre buckets filled with water and a small amount of soap and leave to soak for at least 15 minutes to loosen the soil from the crowns.
    - Move the root crowns back and forth in the water to assist soil removal.
    - If the soil is wet or easily removed from the crowns without damaging the roots, then there's no need to soak and can continue to the next step.
- After 15 minutes or more lift out the root crowns and spray with low pressure water to remove the rest of the soil but be careful not to damage the root system. With bean roots do not spray, instead carefully massage, and move the root ball in water and delicately wash all soil off the roots. Be careful to not loosen nodules from the roots if they are present.

- Leave the root sample in water until it is to be scored, esp. bean roots, as these dry out quickly and then stay in the angles they happen to be in when drying, giving inaccurate results. Leaving them in water will allow the root system to remain as hydrated and unchanged as possible.
- Take care to ensure that the root system is only for one plant. Crops with a high plant population can have plants growing very close to each other.
- Once the roots are free from soil, place each root crown on a flat black surface (e.g., A3 piece of black card) and take a picture from above, keep camera distance and setting consistent between pictures. Then evaluate each root crown individually.

Nodulation

- **Nodulation no.:** Estimation of active nodule number: 1 = greater than 80; 3 = 41 – 80; 5 = 21 – 40; 7 = 10-20; 9 = less than 10 nodules.
- **Nodule size:** Using the dots on the scoreboard (Fig. 10) (millimetres 1 – 5), estimate the average nodule size.
- **Active nodules:** Select and remove 3 nodules (if there are a range of nodule sizes pick representative averaged size nodules) and cut through the centre to check for pink colouration, which identifies if the nodule is active. Record the number of active nodules.
- **Nodulation score:** Provide a score of 1 to 8 to indicate nodule number and pattern of crown nodulation, occurring on the tap root below the seed. Crown nodulation consists of large nodules or tightly packed groups of nodules which completely encircle the tap root close to the seed (Figure 11).

Stem and Root traits

Demonstration video on how to complete the measurements below can be access here.

- **Stem width:** Using callipers (or a ruler if digital callipers are not available) measure the width of the main (largest) stem to the nearest 0.01 mm at soil level root.
- **Tap root diameter:** Measured 2 cm below the Cotyledons.
- **Width of the root system:** Measure, with a ruler, the width of the root system at 1. the widest point and 2. 90 degrees from this point (to assess spread of roots in both directions). The root system may have to be turned about its axis to find this, as the root system dimensions may be asymmetrical due to wider spacing between the rows than within the row.
- **Depth to maximum width:** Measure the vertical distance from the base of the shoot system (position where the soil surface was) to the point of maximum root system width. It may help to use a ruler to define the widest point.
- **Maximum root depth:** Measure the maximum root depth of the root system from the position where the soil surface was.
- **Root system angle:** Using the "Maize Shovelomics Board", record the angle from the horizontal of the outermost roots on both sides of the crown intersect 10 cm arc when root origin is placed at the centre.
- **Outermost epicotyl root length:** Measure the length of the outermost roots (left- and right-hand side of the root crown), using a ruler (Figure 9). Measure the roots that would fall within the arc for angle assessment.
- **Epicotyl root number:** Number of visibly functional epicotly roots**.**

- **Epicotyl root branching density.** Taken from a 2 cm representative segment of epicotyl root: 1 = no root branches; 3 = 1 branch; 5 = 2 branches; 7 = 3 branches; 9 = 4or more branches.
- **Primary root length:** Measure the length of the primary/tap root, using a ruler.
- **No. Primary root laterals:** Count the number of lateral roots coming off the tap/primary root, the lateral roots (Figure 9).
- **Primary root branching density:** Taken from a 2 cm representative segment score the number of lateral roots; 1 = no lateral roots; 2 = 1 lateral root; 5 = 2 laterals; 7 = 3 laterals; 9 = 4 or more laterals.
- **Lateral root branching density:** the number of roots coming off the lateral roots. Record the average score of five representative roots in to one of four classes:
    - Class 4 = > 10 roots per cm lateral root
    - Class 3 = 6 - 10 roots per cm lateral root
    - Class 2 = 1 - 5 roots per cm lateral root
    - Class 1 = < 1 roots per cm lateral root

After the photo and root crown assessments have been completed the root system can be carefully taken apart for the optional assessments listed below. It will also be important to keep the roots from each plant separate and in water ready for scanning with the WinRhizo software.

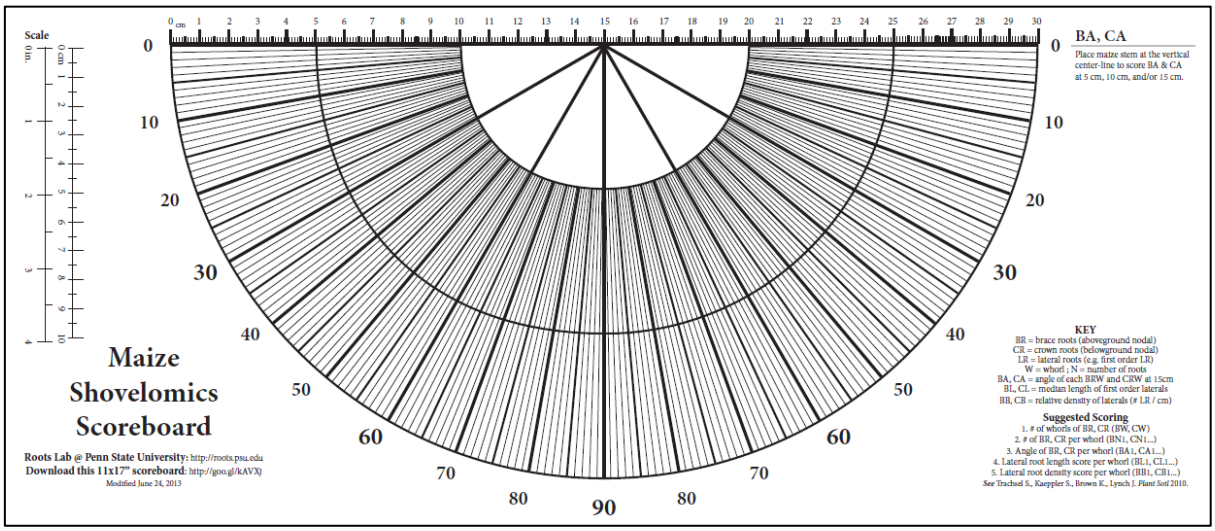

Figure 8. Maize Shovelomics Scoreboard used to record root system angle. To access the scoreboard please use this Board11x17_Maize_Shovelomics_062413JY.pdf. Importantly, instructions for printing to scale found Shovelomics: High Throughput Phenotyping of Root System Architecture — Research — Department of Plant Science (psu.edu) .

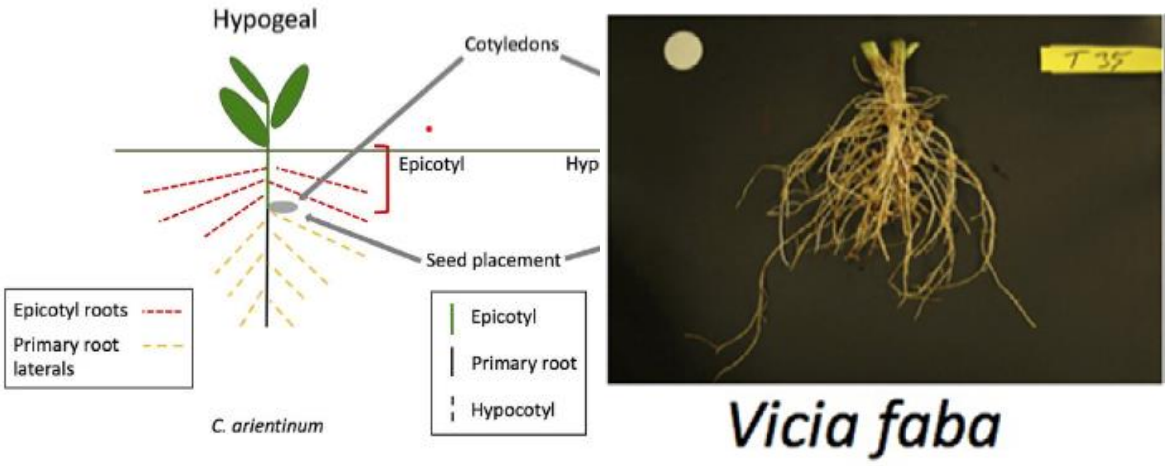

Figure 9. Diagram of a hypogeal type bean plant root systems showing epicotyl, primary and lateral roots. Photo of the faba bean root system.

Figure 10. Example of Bean board, with example nodule sizes, in mm, which needs to be constructed to use for shovelomics assessments.

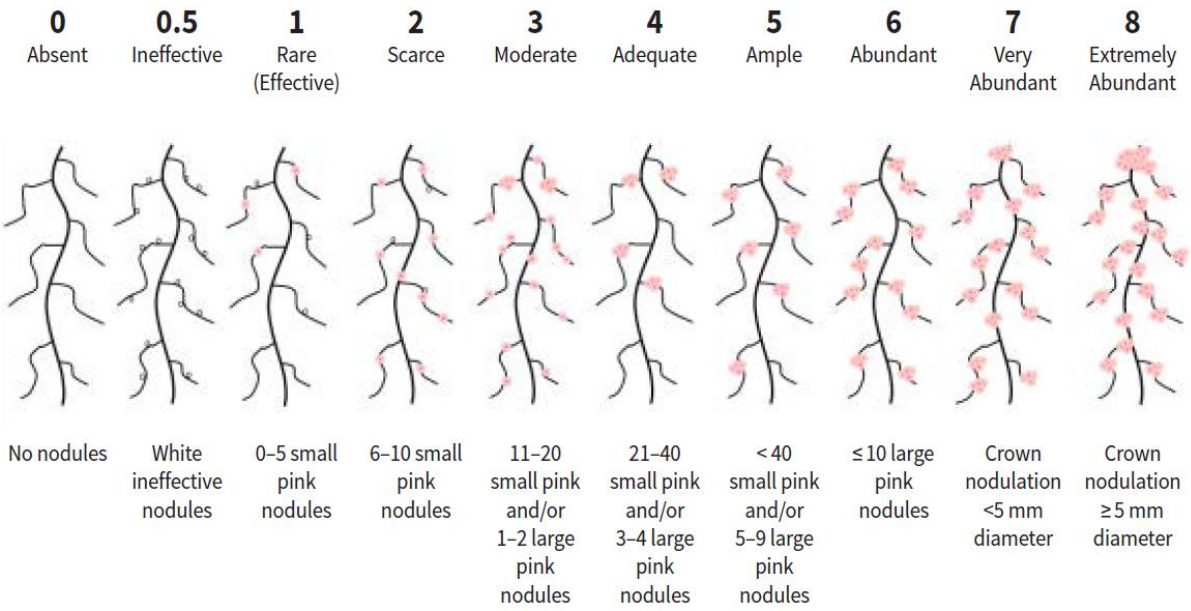

Figure 11. Bean nodule scoring chart.

Root scanning (optional and NOT included in the Root2Res WP2 Experiments)

Once the shovelomics assessments have been completed, separate the above ground biomass from the roots by cutting just above the base of the crown (where the shoot changes from green to white). Next snip the roots off the position where they join the below ground stem. This will give three samples: above-ground shoot biomass, below-ground shoot biomass and roots.

Retain the above-ground and below-ground shoot samples for biomass assessment. Scan the individual root systems of each plant using a flatbed scanner. Place each plant in a shallow tray of water, ~1-2 cm in width, and separate the roots so they are not overlapping each other. Scan this plant in the tray. Analyse the scanned image using WinRhizo software to measure total root length and root diameter.

Biomass assessment (optional and WILL be included in the Root2Res WP2 Experiments)

Dry the roots above-ground-shoot and below-ground shoot samples separately in the oven at 80°C for 48 hours or until no further weight loss then measure root and shoot dry weight. Keep the roots and shoots for each plant separate (in labelled paper bags).

2. Shovelomics Standard Operating Procedure for Barley

Version 2: April 2023

Usually taken in the spring prior to stem extension but can be done at any time. This protocol is an amalgamation of the maize and the wheat shovelomics work at The University of Nottingham, UK. It is designed for cereal crops. It has been tested for wheat and barley. If used for other cereals, it is advised to check that the assessments are suitable. The protocol is based on sampling plants within an experimental field plot trial. If you have any questions or comments, please get in touch.

Authors

Christina Baxter, christina.baxter@adas.co.uk
Charlotte White, charlotte.white@adas.co.uk

Relevant publications

Trachsel, S., et al. (2010). Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil*; doi 10.1007/s11104-010-0623-8.

York, L. M. et al. (2018). Wheat shovelomics I: A field phenotyping approach for characterising the structure and function of root systems in tillering species. *BioRxiv*; doi 10.1101/280875.

Slack, S., et al. (2018). Wheat shovelomics II: Revealing relationships between root crown traits and crop growth. *BioRxiv*; doi 10.1101/280917.

Fradgley, N., et al. (2020). Effects of breeding history and crop management on the root architecture of wheat. *Plant and Soil*; doi 10.1007/s11104-020-04585-2.

Time required

- 0.25 hours to sample 5 plants (single plot) in the field.
- 2.2 hours to measure 5 plants (single plot) in the lab.

Materials and Equipment required for field sampling

- Spade
- Sampling bags
- Marker
- Trial plot map

Materials and Equipment required for lab analysis

- Pen/paper or laptop to record measurements
- Laminated 'Maize Shovelomics Board'
- Black A3 sheet of card
- >10 litre buckets and soap to soak plants
- Camera and tripod
- Ruler
- Tweezers for handling and separating out roots
- WinRhizo software, flatbed scanner, and scanning trays (optional)

- Drying oven, oven trays, filter paper, and scales which measure to four decimal places to measure above/belowground biomass (optional)

Timing of measurement

Measurements can be done anytime from the end of tillering (just prior to stem extension) to flowering. It is best performed when the soil is moist and soft. This may be more likely at earlier growth stages.

Evaluation during the flowering period minimises differences in phenology among lines that may affect root development. Also, at flowering the mature root phenotype is evident.

For the Root2Res project we are targeting the end of tillering for shovelomics assessment.

Excavation of plants

In each plot, excavate five plants from 2 or more sample points in the plot. Depending on planting density, it will probably be necessary to excavate a few plants together. The aim is to extract 5 entire and undisturbed root crowns from each plot. If removing groups of plants, sample at least two groups of plants from two different rows, in two different areas of the plot, avoiding the outer two rows.

- Excavate each plant, or group of plants, using a spade. Select one plant (or group of plants), remove the top 20 cm (or the full length of the spade) of soil around the plant to ensure the full root crown is removed. So that there's a cylinder with 20 cm diameter around the plant.
    - Insert the spade 20 cm deep on either side of each plant, with the width of the spade parallel to the row.
    - Ensure approximately the same volume of soil is removed for each plant sampled.
- Place removed plants, careful not to disturb the soil attached to the crown too much, into a labelled sample bag to transport back to the lab.
    - If some of the soil comes away from the roots it's not necessary to place it in the sample bag, as long as all the plant material is sampled from within the volume of soil removed.

Root crown assessments (in the lab)

Root crowns from the same plot can be assessed together.

- Place the five root crowns from each plot into >10 litre buckets filled with water and a small amount of soap and leave to soak for at least 15 minutes to loosen the soil from the crowns.
    - Move the root crowns back and forth in the water to assist soil removal.
    - If the soil is wet or easily removed from the crowns without damaging the roots, then there's no need to soak and can continue to the next step.
- After 15 minutes or more lift out the root crowns and spray with low pressure water to remove the rest of the soil but be careful not to damage the root system. It may also be helpful to instead carefully massage and move the root ball in water and delicately wash all soil off the roots.

- Leave the root sample in water until it is to be scored. Leaving them in water will allow the root system to remain as hydrated and unchanged as possible.
- Take care to ensure that the root system is only for one plant. Crops with a high plant population can have plants growing very close to each other.
- Once the roots are free from soil place each root crown on a flat black surface (e.g., A3 piece of black card) and take a picture from above, keep camera distance and setting consistent between pictures. Then evaluate each root crown individually:

Cereal plant specific measured traits.

- **No. of tillers:** Count the number of tillers or stems associated with each root crown.
- **Width of the root system:** Measure, with a ruler, the width of the root system at 1. the widest point and 2. 90 degrees from this point (to assess spread of roots in both directions). The root system may have to be turned about its axis to find this, as the root system dimensions may be asymmetrical due to wider spacing between the rows than within the row.
- **Depth to maximum width:** Measure the vertical distance from the base of the shoot system (position where the soil surface was) to the point of maximum root system width. It may help to use a ruler to define the widest point.
- **Maximum root depth:** Measure the maximum root depth of the root system from the position where the soil surface was.
- **Root system angle**: Using the "Maize Shovelomics Board" (Figure 12), record the angle from the horizontal of the outermost roots on both sides of the crown at between 5 cm depth from the shoot base.

*For the following assessments it will be important to identify the nodal (or crown) root system and the seminal root system (*Figure 13*). If both root and shoot biomass are to be assessed keep all parts of the plants. It will also be important to keep the roots from each plant separate and in water ready for scanning with the WinRhizo software, if this is taking place.*

- **Nodal root length:** Measure the length of the outermost nodal roots (left- and right-hand side of the root crown), using a ruler. These are the roots originating from the crown, above the seed. Measure the roots that would fall within the arc for angle assessment.
- **Nodal root branching density:** Branching density is the number of lateral roots emerging from the nodal roots. Record the average score of five representative roots into one of four classes:
    - Class 4 = > 10 lateral roots per cm nodal root
    - Class 3 = 6 - 10 lateral roots per cm nodal root
    - Class 2 = 1 - 5 lateral roots per cm nodal root
    - Class 1 = < 1 branch per cm nodal root
- **No. of nodal roots:** Count the number of nodal roots (roots emerging from the crown).
- **No. of seminal roots:** Count the seminal root number (roots emerging directly from the seed).
- **Seminal root branching density:** Branching density being the number of lateral roots coming off the seminal roots. Record the average score of five representative roots in to one of four classes:
    - Class 4 = > 10 lateral roots per cm nodal root
    - Class 3 = 6 - 10 lateral roots per cm nodal root

- Class 2 = 1 - 5 lateral roots per cm nodal root
- Class 1 = < 1 branch per cm nodal root
-

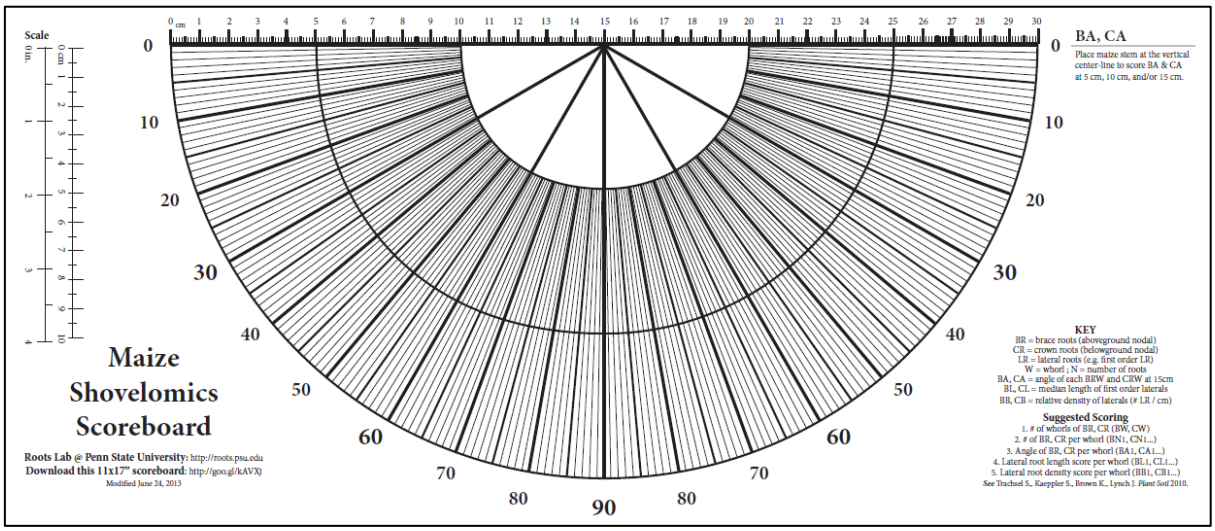

Figure 12. Maize Shovelomics Scoreboard used to record root system angle. To access the scoreboard please use this Board11x17_Maize_Shovelomics_062413JY.pdf..

After the photo and assessments have been completed the root system can be carefully taken apart to assess the following traits. It will be important to identify the nodal root and seminal root system (Figure 13). Both root and shoot biomass are to be assessed, so please keep all parts of the plants. It will also be important to keep the roots from each plant separate and in water ready for scanning with the WinRhizo software.

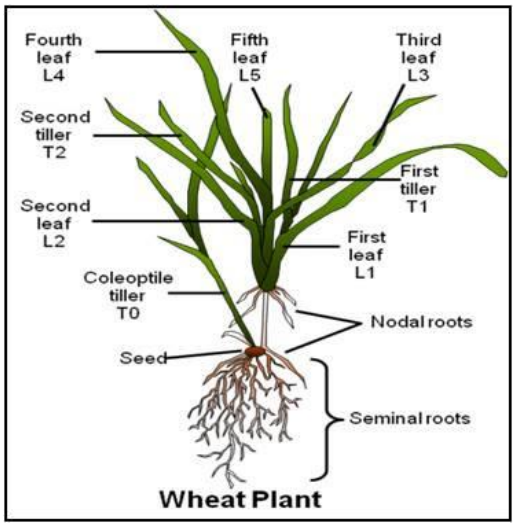

Figure 13. Image of wheat plant showing seminal and nodal root distinction.

Root scanning (optional and NOT included in the Root2Res WP2 Experiments)

Once the shovelomics assessments have been completed, separate the above ground biomass from the roots by cutting just above the base of the crown (where the shoot changes from green to white). Next snip the roots off the position where they join the below ground stem. This will give three samples: above-ground shoot biomass, below-ground shoot biomass and roots.

Retain the above-ground and below-ground shoot samples for biomass assessment. Scan the individual root systems of each plant using a flatbed scanner, before scanning. Place each plant in a shallow tray of water, ~1-2 cm in width, and separate the roots so they are not overlapping each other. Scan this plant in the tray. Analyse the scanned image using WinRhizo software to measure total root length and root diameter.

Biomass assessment (optional and WILL be included in the Root2Res WP2 Experiments)

Dry the roots and above-ground-shoot and below-ground shoot samples separately in the oven at 80°C for 48 hours or until no further weight loss then measure root and shoot dry weight. Keep the roots and shoots for each plant separate (in labelled paper bags).

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Confederaziun svizra
Swiss Confederation
Federal Department of Economic Affairs, Education and Research EAER
State Secretariat for Education, Research and Innovation SERI

3. Shovelomics standard operating procedure for potato

Version 2: may 2023

Usually taken in the late spring/early summer prior to flowering and tuberization. This protocol is an amalgamation of the maize and bean shovelomics work at Penn State University, USA and designed for potato. The protocol is based on sampling plants within an experimental field plot trial. If you have any questions or comments, please get in touch.

Authors:

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Charlotte White, charlotte.white@adas.co.uk
Adapted for potato by Lawrie Brown, lawrie.brown@hutton.ac.uk

Relevant publications

Trachsel, S., et al. (2010). Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil*; doi 10.1007/s11104-010-0623-8.

Burridge J., et al. (2016) Legume shovelomics: High-Throughput phenotyping of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* subsp, *unguiculata*) root architecture in the field. *Field Crops Research*; doi 10.1016/j.fcr.2016.04.008.

Burridge JD, Rangarajan H, Lynch JP. Comparative phenomics of annual grain legume root architecture. Crop Science, 2020;60:2574-2593

Time required

- 0.25 hours to sample 5 plants (single plot) in the field
- 2.2 hours to analyse 5 plants (single plot) in the lab

Materials and Equipment required for field sampling

- Spade
- Sampling bags
- Marker
- Trial plot map

Materials and Equipment required for lab analysis

- Pen/paper or laptop to record measurements
- Laminated 'Maize Shovelomics Board'
- Black A3 sheet of card
- >10 litre buckets and soap to soak plants
- Camera and tripod
- Ruler

- Callipers
- Tweezers for handling and separating out roots
- WinRhizo software, flatbed scanner, and scanning trays (optional)
- Drying oven, oven trays, filter paper, and scales which measure to four decimal places to measure above/belowground biomass (optional)

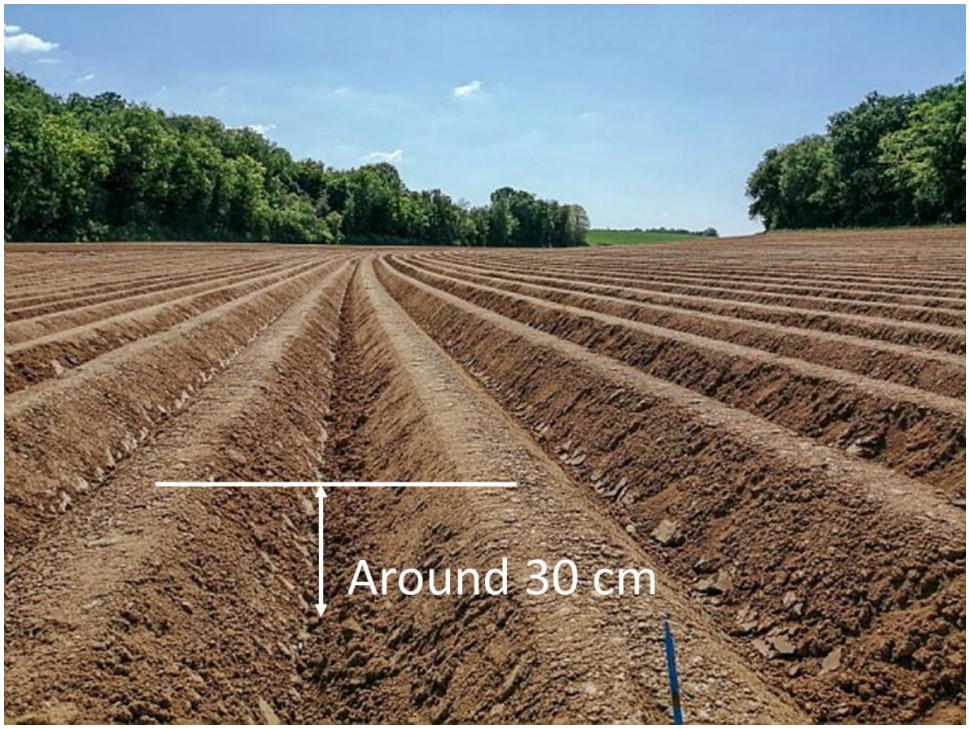
Timing of measurement

Measurements prior to flowering and tuberization (approx 3 to 4 weeks after emergence = 30 DAE). It is best performed when the soil is moist and soft.

Excavation of plants

In each plot, excavate five plants from a range of sample points in the plot. The aim is to extract 5 entire and undisturbed root crowns from each plot.

Excavate each plant using a spade. Select one plant, remove the top 30 cm (or the full length of the spade) of soil around the plant to ensure the full root crown is removed.



So that there's a cylinder with 30 cm diameter around the plant. Ensure the mother tuber is attached.

- Insert the spade 30 cm deep on either side of each plant, with the width of the spade parallel to the row.
- Ensure approximately the same volume of soil is removed for each plant sampled.

Place removed plants, careful not to disturb the soil attached to the crown too much, into a labelled sample bag to transport back to the lab.

- If some of the soil comes away from the roots it's not necessary to place it in the sample bag, as long as all the plant material is sampled from within the volume of soil removed.

Root crown assessments (in the lab)

Root crowns from the same plot can be assessed together.

- Place the five root crowns with mother tuber still attached from each plot into >10 litre buckets filled with water and a small amount of soap and leave to soak for at least 15 minutes to loosen the soil from the crowns.
    - Move the root crowns back and forth in the water to assist soil removal.
    - If the soil is wet or easily removed from the crowns without damaging the roots, then there's no need to soak and can continue to the next step.
- After 15 minutes or more lift out the root crowns and spray with low pressure water to remove the rest of the soil but be careful not to damage the root system.
- Leave the root sample in water until it is to be scored. Leaving them in water will allow the root system to remain as hydrated and unchanged as possible.
- No Plantlet : count the number of plantlet per plant
- Select **the main plantlet (largest)** for analysis and detach from the mother tuber
    - Once the roots are free from soil place each selected plantlet root crown on a flat black surface (e.g., A3 piece of black card) and take a picture from above, keep camera distance and setting consistent between pictures.
    - Then evaluate each root crown individually.

Stem and Root traits

Demonstration video on how to complete the below measurements can be access : https://plantscience.psu.edu/research/labs/roots/projects/finished-projects/usaid-crb/resources/english/shovelomics-videos.

- **Stem width:** Using callipers (or a ruler if digital callipers are not available) measure the width of the main (largest) stem to the nearest 0.01 mm at soil level root.
- **Width of the root system:** Measure, with a ruler, the width of the root system at 1. the widest point and 2. 90 degrees from this point (to assess spread of roots in both directions). The root system may have to be turned about its axis to find this, as the root system dimensions may be asymmetrical due to wider spacing between the rows than within the row.
- **Depth to maximum width:** Measure the vertical distance from the base of the shoot system (position where the soil surface was) to the point of maximum root system width. It may help to use a ruler to define the widest point.
- **Maximum root depth:** Measure the maximum root depth of the root system from the position where the soil surface was.
- **Root system angle:** Using the "Maize Shovelomics Board", record the angle from the horizontal of the outermost roots on both sides of the crown intersect 10 cm arc when root origin is placed at the centre.

Mandatory

- Stolon Number/lenght/weight
- Basal root No/L/wt
- Plantlet No
- Biomass assessment (slide biomass)

Optional

- Secondary stolon No/L/wt
- Node No
- Stolon node root No
- Stolons root No/L/wt

- **Outermost basal root length:** Measure the length of the outermost roots (left- and right-hand side of the root crown), using a ruler (Figure 15).
- **Basal root number:** Number of visibly functional basal roots**.**
- **Basal root branching density.** Taken from a 2 cm representative segment of epicotyl root: 1 = no root branches; 3 = 1 branch; 5 = 2 branches; 7 = 3 branches; 9 = 4 or more branches.
- **Basal root length:** Measure the length of the longest basal root using a ruler.
- **No. stolon root laterals:** Count the number of stolon roots (where the stolons join the stem) (Figure 15).
- **Stolon root branching density:** Taken from a 2 cm representative segment score the number of lateral roots; 1 = no lateral roots; 2 = 1 lateral root; 5 = 2 laterals; 7 = 3 laterals; 9 = 4 or more laterals.
- **Stolon root branching density:** the number of roots coming off the lateral roots. Record the average score of five representative roots in to one of four classes:
    - Class 4 = > 10 roots per cm lateral root
    - Class 3 = 6 - 10 roots per cm lateral root
    - Class 2 = 1 - 5 roots per cm lateral root
    - Class 1 = < 1 roots per cm lateral root

After the photo and root crown assessments have been completed the root system can be carefully taken apart for the optional assessments listed below. It will also be important to keep the roots from each plant separate and in water ready for scanning with the WinRhizo software.

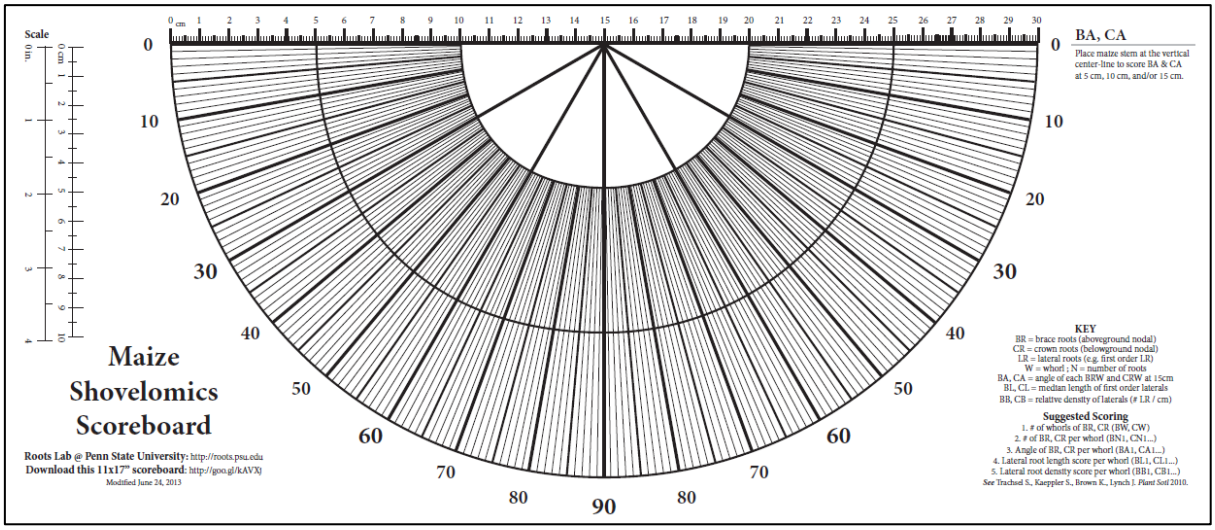


Figure 14. Maize Shovelomics Scoreboard used to record root system angle. To access the scoreboard please use this Board11x17_Maize_Shovelomics_062413JY.pdf. ***Importantly, instructions for printing to scale found*** *https://plantscience.psu.edu/research/labs/roots/methods/field/shovelomics .*

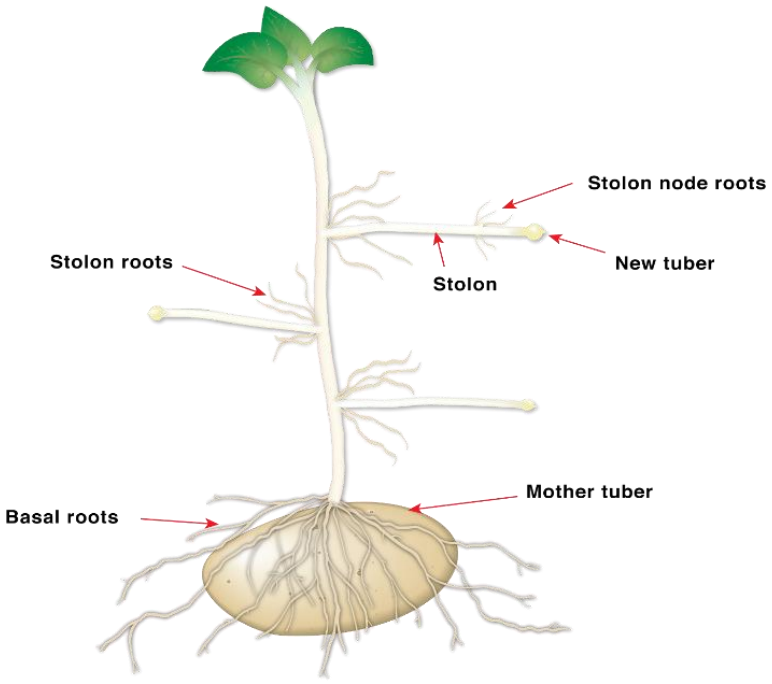


Figure 15. Diagram of a potato plant with root system.

Root scanning *(optional and NOT included in the Root2Res WP2 Experiments)*

Once the shovelomics assessments have been completed, separate the above ground biomass from the roots by cutting just above the base of the crown (where the shoot changes from green to white). Next snip the roots off the position where

they join the below ground stem. This will give three samples: above-ground shoot biomass, below-ground shoot biomass and roots.

Retain the above-ground and below-ground shoot samples for biomass assessment.

Scan the individual root systems of each plant using a flatbed scanner. Place each plant in a shallow tray of water, ~1-2 cm in width, and separate the roots so they are not overlapping each other. Scan this plant in the tray. Analyse the scanned image using WinRhizo software to measure total root length and root diameter.

Biomass assessment (WILL be included in the Root2Res WP2 Experiments)

Separate:

- the above-ground-shoot samples: total shoot (green)
- the below-ground shoot samples:
  - Underground stem (white)
  - Stolons (stem)
- the tubers
  - Mother tuber
  - New tubers
- the roots
  - Stolon roots
  - Stolon node roots
  - Additional roots (for total root weight)
  - Basal roots

Dry the roots, the tubers, the above-ground-shoot and the below-ground shoot samples separately in the oven at 80°C for 48 hours or until no further weight loss then measure root and shoot dry weight. Keep the roots and shoots for each plant separate (in labelled paper bags).

4. Soil Coring standard Operating procedure and Root Analysis of Field Sampled Roots

Version: original version from ADAS

Usually undertaken at mid to post anthesis as this is when the root size is at its maximum, but this sampling can be done anytime between post-anthesis and immediately after harvest if it is important to minimize crop damage. However, root coring can be carried out at any time to assess rooting. The protocol is based on sampling plants within an experimental field plot trial, a different approach is recommended for line/tramline trial sampling. If you have any questions or comments, please get in touch.

Authors

Christina Baxter, christina.baxter@adas.co.uk
Charlotte White, charlotte.white@adas.co.uk

Relevant publications

Clarke, C. K., et al. (2017). Quantifying rooting at depth in a wheat doubled haploid populations with introgression from wild emmer. *Annals of Botany*; doi 10.1093/aob/mcx068.

White, C. A., et al. (2015). Root length densities of UK wheat and oilseed rape crops with implications for water capture and yield. *Journal of Experimental Botany*; doi 10.1093/jxb/erv077

Time required

- 1 hour per plot for collecting soil cores.
- 4.25 hours per plot for washing and scanning cores (based on 5 sampling depths per plot: 0-20, 20-40, 40-60, 60-80, 80-100 cm).

Materials and Equipment required for soil cores

- Hydrocare MCL2 soil sampler (plus ATV or tractor for towing if appropriate) o Check you have auger, scoop, spatula, locking pins (all supplied with the machine)
- Soil core of at least 2.6 cm diameter
- Sampling bags
- Marker
- Cool box
- Trial plot map

Materials and Equipment required for root washing and scanning

- Root washing system (ADAS have a Delta-T root washing system)
- Pre-labelled containers for roots to be placed in
- Recording sheets and pen
- Tweezers
- White plastic trays
- Oven trays
- Filter papers

- Drying oven
- Scales to four decimal places
- WinRhizo software installed on a computer
- WinRhizo root scanner (flatbed scanner)
- WinRhizo scanning trays

Collecting soil cores

Measure and mark the relevant sampling depths on the sampler/auger. Either:

- on the frame of the soil sampler behind the auger, so when the core is extracted, it is easy to see at a glance which are the relevant depths. If this is the method used, care should be taken to ensure the auger is adjusted to the correct height before samples are taken.
- or etch the depth marks on to the auger itself, although this will weaken the auger if it is made from hardened steel rather than heat treated steel. If this is the method used, occasionally check the auger length as exceptionally stony ground will erode the end of the auger.

On arrival at the site, erect the mast on the soil sampler and insert the securing pin, as per manufacturer's handbook. Insert the soil sampling auger and secure with the half round pin as supplied or a nut and bolt securely tightened. Drive up to the first plot and stop at the first sampling point. Lower the mast until the foot plate is at ground level, ensuring the wheels on the soil sampler remain level on the soil surface.

Gently lower the auger to ground level and begin to lower the auger into the soil taking care to avoid "empty hammering" as this will damage the auger. When the auger is approximately at half the required depth, rotate gently then carry on to the required depth. In stony or extremely dry soils, this will take time. At the required depth, again rotate the auger before extracting it. N.B. On some soil types it has been found necessary to rotate the auger at the same time as extracting it, to be certain of consistently retrieving whole cores.

Record the crop growth stage in the trial diary along with the size of the core used and the number of cores taken per plot – this is used to calculate the soil volume sampled.

Take 6 cores per plot with half of the cores sampled in the rows or next to a plant and half taken between the rows, or equidistant between plants. Avoid the outer 2 rows for sampling. Do not sample over a plant.

Where ground conditions permit, the corer should be inserted down to 100 cm before being lifted out of the ground and sub divided into 20 cm horizons. Where this is not possible each 20 cm depth can be removed in turn.

Before taking the soil core brush/remove as much debris (straw etc.) as possible from the soil surface so that it is not sampled. This will save a lot of time after washing when the roots are separated from the organic debris.

For each 20 cm depth, a full soil/root cylinder should be collected. In the event of part of the core being irretrievably lost on extraction, the core should be discarded and another one taken. If any of the 20 cm horizons contains a cavity of greater than 2-3 cm caused by a crack, then the core should be taken again. Smaller cavities can be ignored.

Cores from each 20 cm depth should be placed in labelled bags and into cool boxes to prevent rapid degradation of the samples, and frozen once back on site.

Washing soil cores

Wash cores from the same soil depth and plot at the same time whenever washing multiple samples. Wash cores from all horizons: 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, and 80-100 cm.

Remove soil cores from the freezer prior to washing, allow soils to thaw. Once removed aim to complete each sample within 24 hours to prevent sample degradation. Check the root washer is set at the correct pressure, sufficient to create a vortex in the buckets, by switching the root washer on and opening/closing the pressure release tap on the pump to ensure that the water level is no closer than 0.5 cm from the top of each bucket.

Check the filters (550 micron) under the buckets are clean and free from holes then add the samples to the water buckets. Place the sample bag relating to each sample around the appropriate bucket handle for reference. To assist root recovery, carefully position any soil aggregates in the buckets by hand and hold near water jets at base of bucket or carefully manipulate to ensure all roots are released. Check hands each time they are removed from the washing bucket to ensure no roots are removed or transferred from one bucket to another. Prior to completion of the core washing, turn off pump; ensure no roots are floating to the surface of the bucket or on the sides of the buckets.

Remove the roots that have collected in the filter. To do this, remove the filter and wash (with water) the roots into a pre-labelled container. The roots should be stored in water. Replace the filter and switch root washer back on, check for roots after 5-10 mins, repeat this process until no roots remain in sample.

When root washing is complete, empty all the filters carefully, collecting all the roots by washing the filters with water, and collecting the root samples in pre-labelled containers. The roots should be stored in water. Empty soil and water out of bucket into water reservoir, check no roots have got caught around the water jets. The root washer needs to be emptied, cleaned out and refilled after approximately 8 – 12 plots. Once collected, samples should be scanned within 24 hours to prevent sample degradation.

Root scanning

Prior to scanning, any previous crop debris and non-root material should be carefully removed from the sample. Place root samples on to white plastic trays and separate the root samples from the organic debris and dead roots with tweezers, place clean root samples in labelled containers with water for scanning.

The following instructions have been modified from the WinRhizo manual. They are general guidelines for assessing these types of root samples only. Please also be advised that the WinRhizo software has the capacity to measure a number of parameters and different types of samples, please check the user manuals for further information.

1) Turn on the scanner, followed by the computer.
2) Open the WinRhizo software using the yellow icon on the desktop.

3) When prompted select the default setting, for scanner. Click OK.
4) Choose the scanner you are using from the menu.

Scanning samples

The root samples should have been washed clean and free of debris.

1) The WinRhizo positioning system plates should be used for measuring field/free root samples. Select an appropriate size tray (e.g., 20 cm wide, 25 cm long) and place it on the scanner. Check the scanner instruction manual if anything about this is uncertain.
2) Place the samples in the plates (positioning system) on the scanner, keep the scanner clean and dry as water and debris on the scanner will reduce scan quality.
3) Space roots out evenly and away from the edges of scanning tray.
4) Close the scanner lid carefully.
5) In the image menu: Acquisition Parameters, in the window select: a) image type: grey levels; b) resolution (dpi): high 600. c) dust removal: low; d) delect the regent positioning system; e) select the size of tray used: e.g., with tray 20 cm wide and 25 cm long and f) OK.
6) Acquire image using the large button with the picture of a scanner in the top left of the screen. This should take 5 minutes.

Image analysis settings

1) When scanned fit the picture to size so all roots can be seen, button on bottom of screen left-hand side.
2) In the analysis menu: roots and background distinction.
3) Click based on grey levels.
4) Manual - this value will need to be altered to capture as much of the root system as possible, for very fine roots this may need to be as high as 200. Once a manual level is selected, it should be fine for all trays scanned that day.
5) To check that the manual selection is picking up the roots, in the background and distinction window, there is a button which toggles between the original and the pixelated image. Use this to set the best manual setting.
6) Once selected, click OK.

Image analysis

1) Click on the image in the top left-hand corner of the image, click and drag a square to capture roots.
2) A dialogue box will pop up. Name the sample e.g., plot 1 0-20 cm, add your operator initials.
3) The image will be analysed, showing red- and yellow-coloured roots, resize analysed area, if necessary, hold mouse over green edge of square to get a double headed arrow and resize accordingly in or out, this will automatically reanalyse the selection.
4) Once the analysis is complete the data is ready to be saved, you will need to open or create a data file.
5) At the end of the scanning session transfer all data files to a USB stick and save these to the Local Area Network (LAN).
6) At the end of each scanning session ensure the scanner bed is clean and dry before switching everything off.

Dry weights

After scanning, record the dry weights of the sample, recording the weight to four decimal places. The fresh weights are not recorded as the root samples are wet and this would affect the measurements.

Weigh the tins and record weights on record sheets. Dry weights can be carried out using the vacuum system on filter papers or other appropriate method. If drying in an oven, dry at 80^{o}C for 48 hours or until no further weight loss and record the dry weight. Use a foil lined tin to measure and dry the roots, there should be sufficient foil to create a cover/lid over the root material to prevent any fine roots being dislodged from the tin.

Data analysis/traits measured

Analysis of the root samples from the soil cores using WinRhizo will measure the traits total root length and **root diameter**. The **root length density** (cm/cm^{3}) of the crop in each soil horizon can be calculated using the total root length and the volume of soil the roots were sampled from.

Root dry weight measurements can be used to calculated **root biomass** (mg/cm^{3}) in each soil horizon using the total root biomass and the volume of soil the roots were sampled from.

5. Minirhizotron Standard Operating Procedure

Version: original version from ARVALIS

Authors

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Relevant Publication

Hansson, A.C. and Andrén, O. (1987) 'Root dynamics in barley, lucerne and meadow fescue investigated with a mini-rhizotron technique', Plant and Soil, 103(1), pp. 33–38. Available at: https://doi.org/10.1007/BF02370664.

Lebègue, C. et al. (2004) 'Mise en œuvre de carottages de sol et de minirhizotrons pour l'étude à long terme de la réponse des fines racines d'épicéa (Picea abies (L.) Karst.) à l'augmentation de la concentration en CO2 dans l'atmosphère et la nutrition minérale', BASE [Preprint]. Available at: https://popups.uliege.be/1780-4507/index.php?id=13964 (Accessed: 21 March 2023).

Postic, F., Beauchêne, K., Gouache, D., Doussan, C., 2019. Scanner-Based Minirhizotrons Help to Highlight Relations between Deep Roots and Yield in Various Wheat Cultivars under Combined Water and Nitrogen Deficit Conditions. Agronomy 9, 297. https://doi.org/10.3390/agronomy9060297.

Installation of Minirhizotron tubes

PURPOSE AND FIELD OF APPLICATON

Procedure for installing the equipment necessary for root monitoring by endoscopy, known as a "minirhizotron".

PRINCIPLE

Drill a hole in which a transparent tube is installed. This tube accommodates the root endoscope at each measurement session. Particular attention should be paid to the angle, to the correct adjustment of the diameter and to the emerging part of the tubes to obtain a good device, prevent any risks of inducing bias.

MATERIALS AND CONSUMABLES

Manual drilling

- 45° angle auger support
- 2m long auger, diameter 70mm
- 3m long auger, diameter 70mm
- Knife (for emptying gouges)
- Rack (to remove soil)
- Wire (or other means of securing the auger to the support)
- (cutter)
- (marker)

Tubes

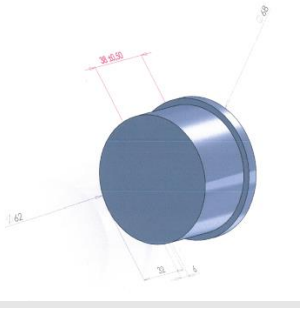
- Transparent extruded PMMA tube, length 2m, diameter 70mm

Product reference: Extruded transparent PMMA 7cm internal diameter, Extruded transparent PMMA tubes 70x64 length 2000mm

Supplier: AbaquePlast, 41 avenue Gaston Monmousseau, 93245 Stains Cedex - France

Cost: 15€ per m (30€ for a 2 m tube)

- PVC cap (glued to the bottom of the tube)
  PVC cap to be done yourself, possible support can be brought by ARVALIS' (France)



Dimensions:

Finishing the installation

- Reflective thermal insulation on a roll
- Chatterton / scotch tape
- Vinyl cap
- Insulating foam tube

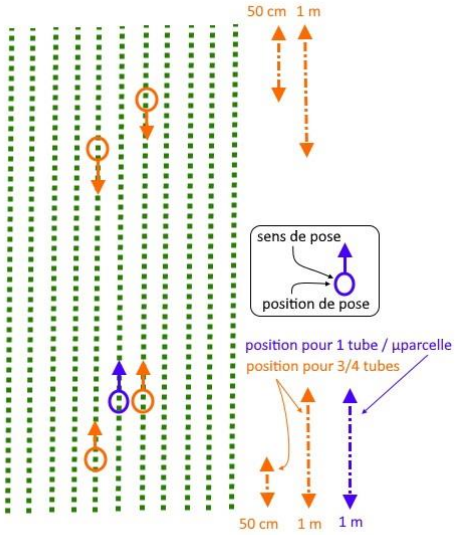
CONTENT OR CONDUCT OF OPERATIONS

Choice of location

The tubes should preferably be placed in the middle row, in the direction of the row. In addition, to minimize trampling during regular operations, it is preferable to drill 1 m from the edge of the experimental plot (possible trampling limited to the edge zone).

The position of the tubes in the microplot will depend on the protocol requirements (1 or more tubes per plot) of the crop and the access and packing conditions.

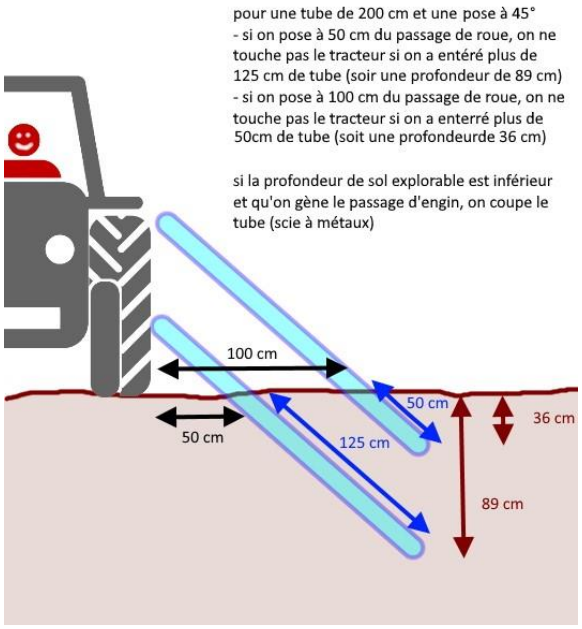
Figure 16 below shows the distribution of tubes for an 11-row cereal trial with access possible on the first plot on either side.



Proposition de positionnement des tubes pour l'essai 40 B Minirhiz - Méthodo

Figure 16. T*he distribution of tubes for an 11-row cereal trial with access possible on the first plot on either side. Sens de pose : Orientation of the installation, Position de pose : Position of the installation, Position pour 1 tube/microparcelle: position for 1 tube/microplot, Position pour 3/4 tubes : position for 3/4 tubes*

Be careful with the depth of installation, because if the tube protrudes too far from the ground, it can become an obstacle to the passage of machinery.



For a 200cm tube and a 45° installation:

- if the tube is laid 50cm from the wheel arch, it does not touch the tractor if more than 125cm of tube is buried (i.e., a depth of 89cm).
- if you lay at 100sm from the wheel arch, you do not touch the tractor if you have buried more than 50cm of tube (i.e., a depth of 36cm).

If the depth of the ground to be explored is less and the tractor's passage is hindered, the tube must be cut (with a hacksaw).

Drilling

Place the 2 m auger on the 45° support and position the assembly to obtain the desired alignment (see "choice of location").

At this stage, 2 operators stand on each side of the 45° support, one foot on the base of the object, to keep the assembly stable. Attach the auger to the support with wire (or other means) to keep it on the support and prevent it from lifting.

The 3rd operator can start drilling. Be careful not to produce too large holes (think of the tube/earth contact), so reduce the number of auger turns as much as possible (typically 1-1.5 turns are sufficient). Once the maximum depth of the 2 m auger is reached, repeat the operation with the 3.5 m auger.

Installation of the tubes

Take a clear PMMA tube (unpacked).

Insert the plug into the hole at the bottom of the tube. Ideally, the drill sticks along the entire length of the tube.

When drilling to the maximum depth, 10 to 15 cm of the tube will protrude from the ground.

Finalization

Wrap and tape a reflective thermal insulation strip around the exposed part.

Leave 2 cm around the neck (to be able to cap it later).

Place a mark at 0H on the inner wall (tape) to be used as a marker for the insertion of the endoscope.

Note the tube number on this tape.

Close the endoscope with the cap and its insulating foam tube.

Read the length of the tube above ground and fill in the file [to be determined-> a file "n°trial.xls" in the data directories for processing]. The following columns must be filled in at least.

- The trial test number
- The tube number
- Micro plot number
- The length above ground
- Variety
- Modality
- Repetition

Removal of the tubes

At the end of the season (before harvest), it is necessary to remove the tubes. Depending on the soil conditions, it is possible to:

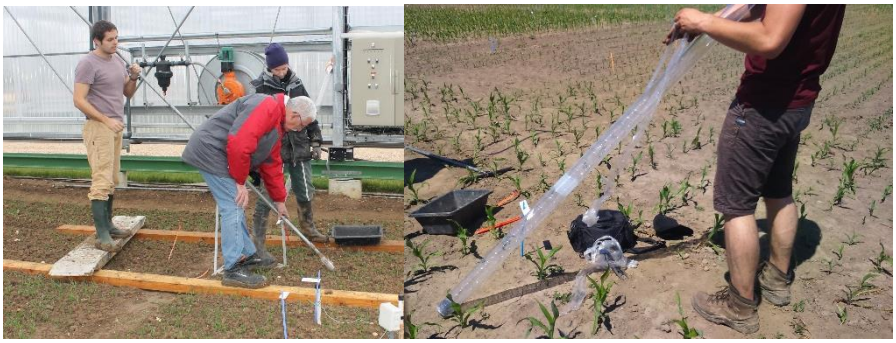
- Water the soil around the tube thoroughly, rotate the tube to loosen the soil-tube contact, remove the tube without disturbing the soil too much.
- Alternatively, insert a plastic tube inside the tube and lift with a mini-excavator, in which case the plants monitored during the campaign will be removed.

FIDELITY

The tubes should be laid at 45° from the vertical and not at 45° from the ground (which may slope), the slope of the ground must be measured in the direction of the plot if it is significant (greater than 5% corresponding to a 12 cm depth estimation error in the case of two tubes in the opposite direction of installation).

SELF-MONITORING AND TIME REQUIRED

The tubes are installed with an auger by at least 2 people.



Feedback from ARVALIS's research station in 2018

- (dry and stony soil) Installation of 14 tubes in 3 days with 3 people => **1h / tube / 2 people**
- (deep soil) Installation of 8 tubes in 1 day with 2 people => **30 min / tube / 2 people**

Feedback from ARVALIS's research station with the intervention of a farmer service company in 2020-2021

- Installation of 108 tubes in 27 h by 2 people => **15 min / tube / 2 people**
- Service cost ~2500€ per site (located in southern France)

Feedback from ARVALIS's research station with the use of the drill built in the Plant2pro MINIRHIZ project in 2020.

- Installation of 8 tubes in 1h by 3 people => **8 min / tube / 3 people**

Root endoscopy measurements in field

PURPOSE AND FIELD OF APPLICATON

Root image acquisition procedure using a root endoscope placed in a minirhizotron.

PRINCIPLE

A rotating scanner (sometimes called a camera) is inserted into the bottom of the tube where it will image the entire circumference of the tube to a height of 24cm. The scanner is then raised by one scan height (using dedicated rings on the rod holding the scanner) and the measurement is repeated until the surface horizon (an all-white area) is seen on the images.

Root endoscopy image acquisitions should be preceded by a calibration after each restart of the instrument (or every 6 tubes) and should be correctly named to facilitate root detection and root profile reconstruction. Current endoscopes (CI-600 from CID Inc., 2017) are powered and controlled via a computer (laptop or tablet, running Windows).

MATERIALS AND CONSUMABLES

- CI-600 (cf. instructions for the device):
    - Endoscope
    - Calibration tube (white coated tube)
    - USB cable (+ USB-miniUSB adapter for tablets) 70mm
    - Metal rods (aluminium or steel) to guide the endoscope
    - Rigid cap with holes
    - Cloth (green)
    - Allen key ("small")
    - Instructions for the device
- Laptop or tablet :
    - Windows XP-32 bits or Windows 7 -9 -10 32/64 bits
    - CID software installed
    - Working computer/tablet battery
- Availability of a NAS in the station:
    - Access test validated

Remark: It is possible to plan to move all the equipment with a wheelbarrow and to use 2 pennants to support the camera rod.

The installation software is available on the website of the company producing the scanner.

https://cid-inc.com/support/CI-600/software/

The scanner control software can be installed on a computer or a tablet. Be careful to check the operating system version and the disabling of the software signature check which is well detailed in the installation PDF also available on the provider's website.

CONTENT OR CONDUCT OF OPERATIONS

Preliminaries

Make sure that none of the tubes are filled with water, right to the bottom, as the endoscope is not submersible (2000€ repair). Use the rods and cloth to check for moisture and wipe off any traces of moisture.

Ensure that the computer/tablet battery is fully charged (or at least sufficiently so - the endoscope consumes about 23W during scanning).

Mounting the system

Follow the instructions for the device for details and photos.

Attach the USB cable (and tighten it with the Allen key), then pass it through the large opening in the hard cap.

Slide the aluminum rod through the small opening, screw the end of the rod onto the endoscope.

Place the endoscope in the calibration tube (white tube)

CAUTION: the first models of rods (in aluminum) have fragile screw threads (32 threads per inch) and are therefore easily deformed during assembly/screwing. Ideally, assemble the rod once and for all by screwing in all sections.

Start the computer/tablet, attach the USB cable (take care to keep it in place during handling). Start the "CID" program (green sheet, icon on the desktop).

Settings and calibration

For the following operations, refer to the unit's manual, the interface varies between models.

Set the format of the acquired images to **JPG - colour - 300 DPI** (images of less than 300 DPI will be almost unusable). Select the "filename ICAP Convention" parameter (see below paragraph “File naming”).

Choose the destination directory (ideally "folder_whatever/images_minirhizotron/project_name/session_X/").

Proceed with the calibration with the endoscope in the white tube, launch "calibration".

Once the operation is completed, the device is ready for a measurement session (if it fails, check the USB cable, and start again).

Field measurement

Insertion: Place the endoscope with the 2 white pads facing each other. Insert to the bottom.

First image: Arvalis has decided that the first image ("window 1") is **at the bottom of each tube**, so it is necessary to go up to be in the notch of a stem, so that each photo is quite distinct.

- Select "Scan" (see instructions) and check (or modify) the following fields
- User: initial of the operator in capitals (e.g.. Xavier Dupont will note "XD")

- Session: measurement date format DD.MM.YY
- Tube: Select the current tube number
- Window: Select 1 beginning by the bottom of the tube

REMARK: avoid underscores ("_"), they are used to delimit keys in the ICAP nomenclature for naming image files.

Launch OK, a window opens, once the progress bar advances, the endoscope will start rotating.

Next acquisition: Pull the rod and HOLD the USB cable without pulling it until you reach the next spacer. Change the window number and start an acquisition.

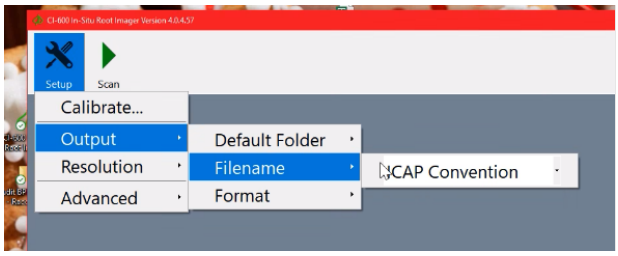
Move to the next tube: Place the endoscope in the calibration tube for travel.

Ending an acquisition session and turning off the power: No special precautions are required. Close the CID program, unplug the device and turn off the computer/tablet. Store in the case

The number of measurements taken in this way will provide an accurate measure of depth.

File naming

The camera control software can take care of the naming of the images as soon as they are created. This means that operators do not normally have to rename the pictures, except in exceptional cases. In order for the automatic naming to work properly, the parameter "filename ICAP Convention" must be selected.



Ensure that the PC or tablet is at the right time and fill in properly:

- the trial number in the 'project' field
- the user's initials in the 'user' field
- the plot number in the 'tube' field (only numbers)
- the window number in the 'Window' field
- [optional] the session number in the 'Sessions' field

By doing this at the time of saving the photo "save", the nomenclature is automatically incremented.

So, if there are any problems with the acquisition, do not hesitate to start again. The new images will have a different name (different time and minute of acquisition). This makes it possible to follow the order in which the images were acquired (useful in the event of a numbering error).

If there are duplicates, be sure to delete the unnecessary images when unloading the data.

Filing and retrieval of raw images

At the end of an acquisition, the images are deposited on the dedicated link to share picture. The technical team will be able to retrieve this file and, after checking the values, report the data in a minirhiz tab.

The data analyse produces the following indicators (in the Depthed file) for each window in each tube (i.e., a distribution by horizons of ~16cm):

- apparent root length (length_mm)
- average diameter of root seen (diam_mm)
- root length density (noted DRL_mm.cm2) = apparent root length / scanned area

For each tube, a distribution per 1 cm horizon of the following indicators is also constructed (in the file trait_with_position):

- colonization profile: % of 1cm*1cm cells with roots at each depth
- density profile: % of root pixels at each depth

PRECAUTIONS FOR USE - SAFETY – MAINTENANCE

Never immerse - non-waterproof equipment: Ensure that none of the tubes are filled with water, using a rod with a cloth around it to wipe the tube clean of any condensation.

Electric security: No electrical risk on this equipment entirely in TBT-S voltage<12V. Fragile usb connectors

FIDELITY

When unloading the images, the images should have soil colours with root traces. The last image of each tube has an all white portion. The first image may also show the image of the tube bottom cap.

Condensation marks, droplets, scratches in the tube do not prevent image analysis. However, heavy condensation inside the tube will damage the scanner. This occurs especially when there are large daily temperature fluctuations.

SELF-MONITORING AND TIME REQUIREMENT

Taking picture**: 15 min /tube /pers**

6. Soil Pit standard operating procedure: Notation of crop rooting

Author

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Relevant publication

Zarzyńska, K., Boguszewska-Mańkowska, D. and Nosalewicz, A. (2017) 'Differences in size and architecture of the potato cultivars root system and their tolerance to drought stress', *Plant, Soil and Environment*, 63(4), pp. 159–164. Available at: https://doi.org/10.17221/4/2017-PSE.

Purpose and Field of application

Evaluate the extent of soil colonization by roots.

Determine the relationship between soil structure and rooting.

Determine a crop's capacity to adapt to the environment (water, soil mineral nitrogen).

All crops, from flowering onwards, to assess definitive rooting.

Principle

Dig a pit.

Note the presence of roots on a vertical face of the soil.

Equipment and Consumables

- Backhoe recommended for digging the pit.
- Spade and shovel
- Rigid blade knife
- Grid (e.g. 80*80 cm) with 2 cm square meshes
- 2 operators in the field

Content or Sequence of operations

Sampling

Choice of plots: place in an area representative of the plot (soil, tillage). In the case of weeded crops such as maize, ensure that the pit and grid are always positioned in the same way in relation to the crop rows.

In general, the pit is about 1 metre wide and 80 cm deep. These dimensions can also be adapted to the scoring objectives.

Number of pits: one per modality is generally used, given the complexity of the measurement. However, soil is not a very homogeneous medium, and two or three repetitions seem necessary to be sure of the conclusions.

Procedure

Open a pit. The observation side is perpendicular to the direction of tillage and sowing. The depth of the pit depends on the type of soil (presence of rocks, etc.). Ideally, it should be 1.2 m for observation at 80 cm. For certain specific applications (soil water reserves exploitable by roots, depth of nitrogen residues to be applied, etc.), a deeper pit may be necessary.

Refresh the observation side with a knife, revealing the roots.

Apply the grid against this face.

Observe the soil horizontally through each square and record on graph paper, the presence or absence of at least one root of the crop in place.

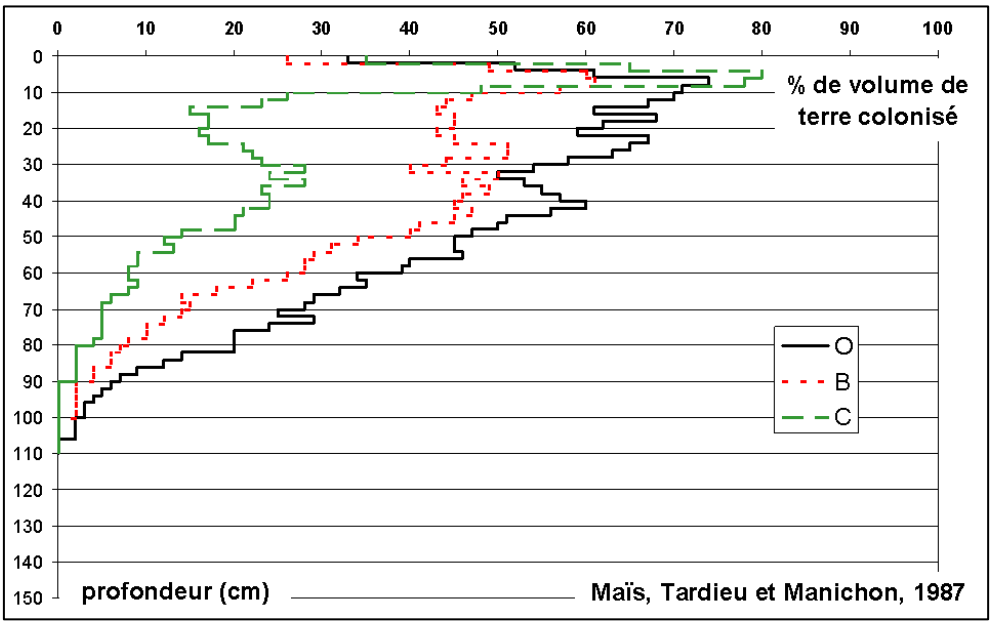
One operator observes the rooting and the second enters the results on a sheet of paper or directly into Excel (computer adapted to the field).

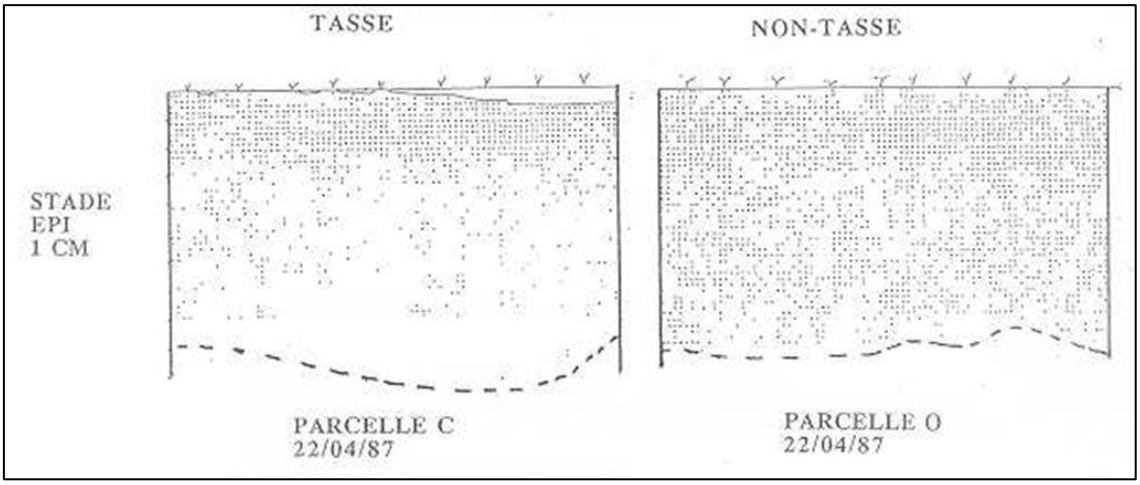
Expressing results

Transcribe the results into an excel table. Results can be coded (0=absence, 1=presence) to express the % of cells colonized for each depth in 2 or 10 cm steps.

Results are presented in the form of:

- a graph ("root profile") showing the % of cells occupied by at least one root for each horizon;
- an image of the actual colonization, represented by the grid in which the cells occupied by at least one root have been blackened.





Operating Precautions - Safety

- The operator must be able to drive and operate a backhoe loader.
- Take precautions to avoid cutting yourself with the knife.

Self-Control and Time Required

For one pit:

- Dig the pit with a backhoe 15 min with 1 person
- Finish the hole with a shovel and prepare the observation face. 15 min with 1 person
- Observe rooting and record on a sheet of paper 30 min with 2 persons
- TOTAL 1 hour 30 minutes (2h if entered in the office)