Root2Res

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

Root phenotyping toolbox for more resilient crops

ARVALIS

Katia BEAUCHÊNE

August 29th, 2024, Rennes FRANCE

Funded by the European Union

Root2Res Mid term symposium

Phenotyping toolbox 01
Context, project description

Root phenotyping methods 02
CE Review

Field root phenotyping 03
Evaluation of different methods

Next steps 04
Update SOP for breeding

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Root2Res Mid term conference

Phenotyping toolbox
Context, project description

01

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Root2Res « Phenotyping toolbox»

Define and provide a complete set of tools to consider root traits

Aims:

Evaluate and improve innovative tools to characterize morphological root traits, microbiome and envirotyping to find useful proxies or methods and bridge the gap between field and controlled environment.

=> IMPROVE methods

WP2 will also coordinate the phenotyping activities of the project to be sure that all trial and data will be available for project analysis.

=> APPLY methods for other WP

Characterize rhizosphere traits focusing on root exudation & associated rhizosphere microbiome

=> DEVELOP methods for rhizosphere traits

Tasks

Phenotyping toolbox for morphological traits (ARVALIS)

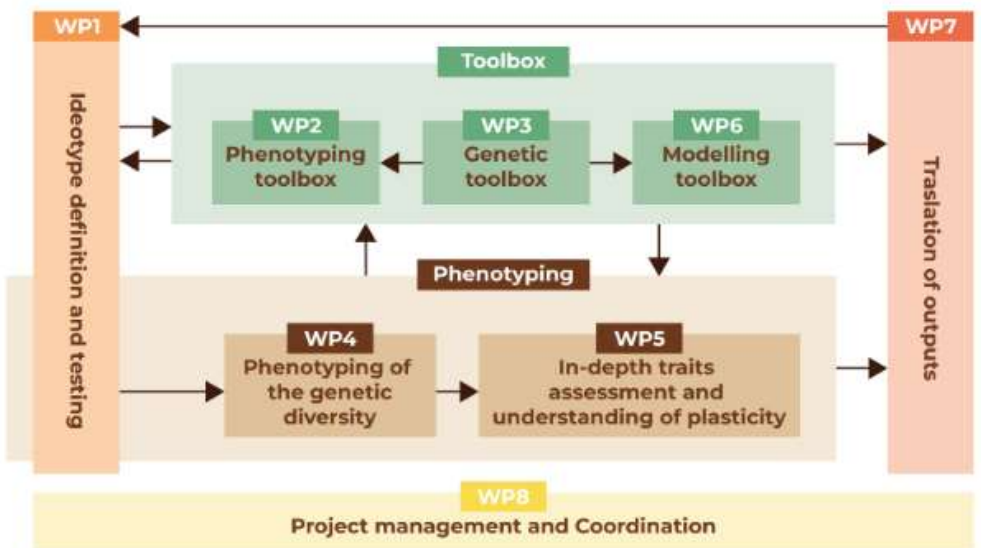
- *Field and Controlled conditions*

Phenotyping toolbox for root and related rhizosphere traits (BOKU)

- *Controlled conditions*

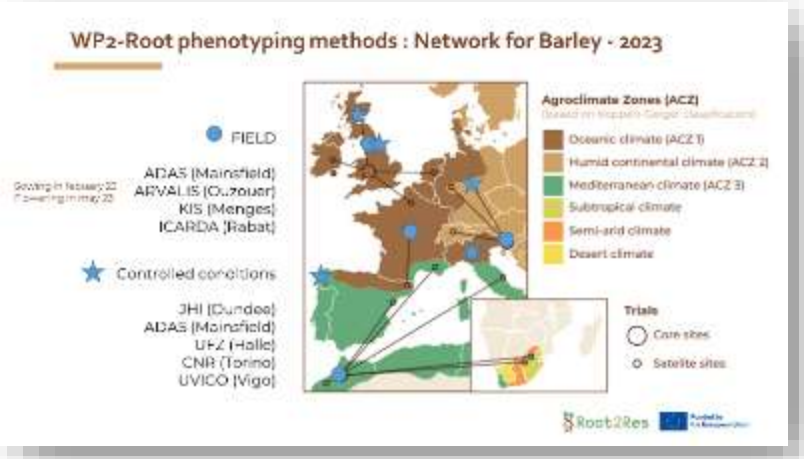
Envirotyping coordination (ADAS)

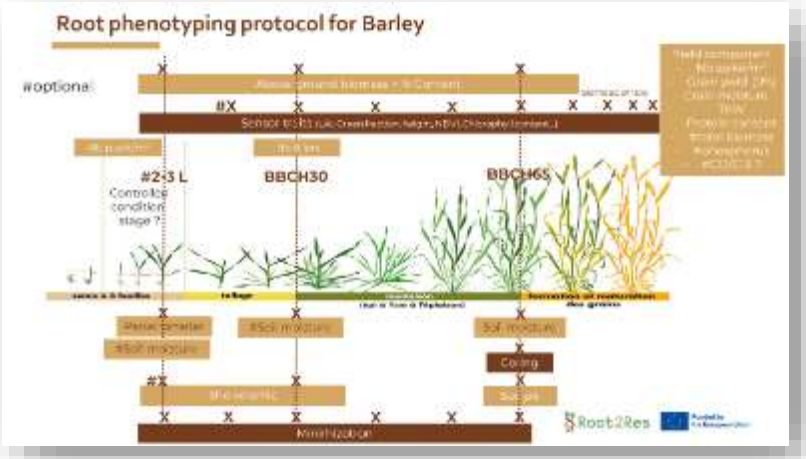
- *Field and Controlled conditions*

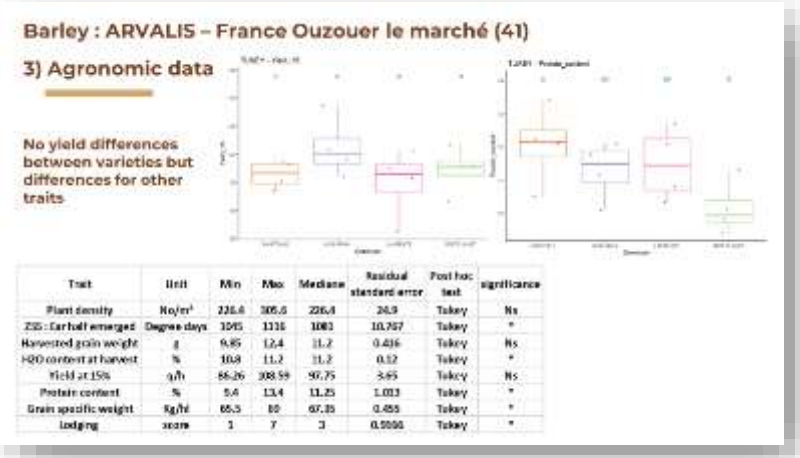


Protocols for each crop

- ✓ Design trial, varieties, seeds, measurement (abg part, roots, environment)…
- ✓ List of mandatory or optional measurement in ProtocolTraits.xlsx
- ✓ TRAINING
- ✓ Sum / overview via some slides
- ✓ Data set format : WP2.1_XXX_23_FP_XXX_1DataTemplate.xlsx
- ✓ First analyses per trial (partner)
- ✓ Share results during 3 crops meetings (cereals, potato, legumes) Field and CE
- ✓ Check and clean data







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Summary of the Root2Res trial network

Location	ACZ	Partner	WPs	2023 Network				
				Cereals		Potato		Faba Bean
				Barley	Durum Wheat	Potato	Sweet Potato	
UK (England)	**1**	**ADAS**	WP2	√ Δ				-
France	**1**	**ARVALIS**	WP2	√		2 x √		
UK (Scotland)	1	JHI, BOKU, UNIDUN	WP2-WP5	Δ		Δ	Δ	Δ
UK (Scotland)	1	JHI	WP2			√ Δ		
Ireland	1	TEAGASC						
Slovenia	**2**	**KIS**	WP2	√		√		√
Germany	2	UFZ	WP5	Δ				Δ
Morocco	**3**	**ICARDA**	WP2	√	√	-		√
Italy	3	CNR	WP2	-				-
Spain	3	IRTA	WP2		-			
Spain	3	UVIGO	WP2	Δ				Δ
France	3	ARVALIS	WP2		√			
South Africa	3	ARC	WP2				√	
Italy	3	UNIVPM	WP2					Δ
Austria	2	BOKU						
Austria	2	BOKU						

WPs	2024 Network				
	Cereals		Potato		Faba Bean
	Barley	Durum Wheat	Potato	Sweet Potato	
WP2	√ Δ				2 x √ Δ
WP4	√				
WP2	√		√		
WP4	√				
WP2					
WP2			√		
WP2			√		
WP2	√		√		√
WP4	√				
WP2					
WP2	√ Δ	√ Δ	√		√
WP4	√				
WP2	Δ				Δ
WP4	√				
WP2	Δ				Δ
WP2		√			
WP2				√	
WP2					
WP5	Δ				
WP5					Δ

Δ Controlled Environment

√ Field

Δ Lysimeter

*√ denotes **field** experiments and Δ **controlled** experiments. Core sites are shown in bold.*

Barley: RGT PLANET, LAUREATE, FAIRYTALE and KWS IRINA
Potato: CARA, DESIREE, INNOVATOR and EERSTELING
Faba bean: LYNX, ZORAN, ASCOT and ELISAR

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Root2Res mid term symposium

Root phenotyping methods 02

CE Review

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Root phenotyping methods

Facilities : how plants are growing ?

Rhizotron (à gauche), rhizotube (au centre), et cultures hydro- ou aéroponiques (à droite) offrent une vue de l'ensemble du système racinaire des jeunes plantes.

Data processing : which traits could be measured ?

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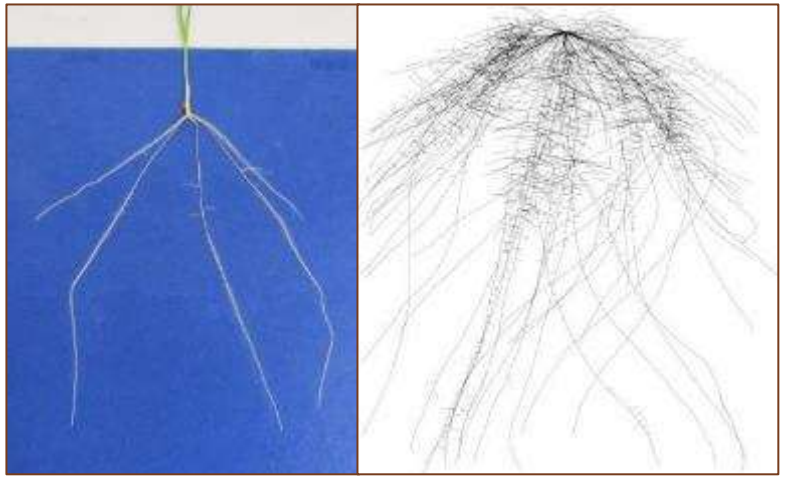
ADAS (UK), Seedling Root Screen

Christina Baxter



Facility description

- Seedling root screen (SRS)
  - Cereals and OSR
  - Plants grown for 2-3 weeks
  - 400 seedling capacity

Specific equipment for root measure

- High quality camera and imaging stand
- Images analysed using RootNav software

Traits measured with specific equipment

- Total root length
- Root length – primary & lateral
- Number of roots - primary & lateral
- Root tip angle - primary & lateral
- Emergence angle – primary & lateral
- Root system width & depth
- Convex hull (Root system area)
- Tortuosity (waviness of growth pattern)

Trials

WP2 2023 CE:

- Spring barley: Seedling root screen (8 var x 20 rep)
- Spring beans TBC: Seedling root screen (8 var x 20 rep)

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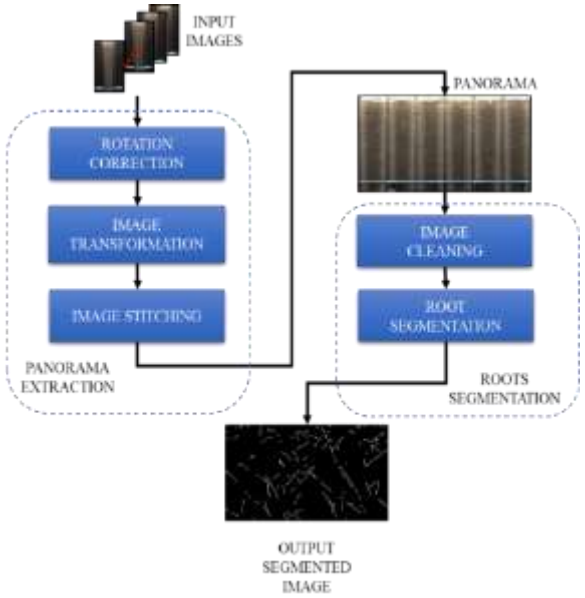
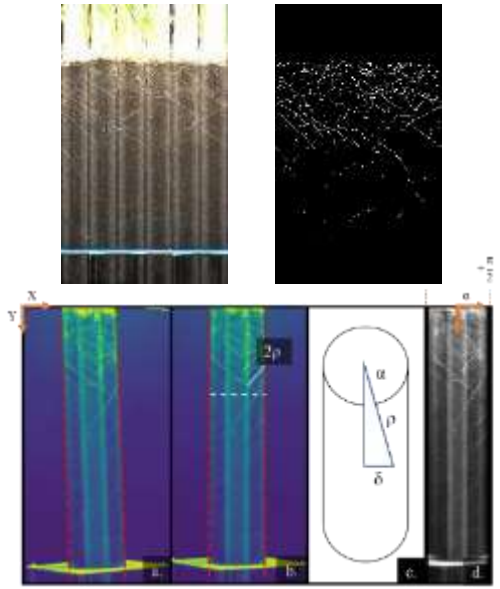
CNR (ALSIA – Metaponto, MT, Italy)

National Research Council of Italy
Institute for Sustainable Plant Protection
PSP

Raffaella BALESTRINI





Facility description

- High-throughput Plant Phenotyping platform (Southern Italy)

Specific equipment for root measure

- Long glass tubes (50 cm)

Traits / Specific equipment

Belowground measure

- Computer vision software pipeline for the analysis of the roots system of a plant
- Multiple shots of a rotating cylindrical rhizotron
- Stitched panorama image
- Roots segmentation

Aboveground measure (if needed)

- RGB, NIR, Fluorescence (top and side)

Trials

Core : Faba bean, controlled conditions
WP2-2023 Methodology 4 genotype x 4 rep (x2 pots for destructive measures) x IRR/N
Long tubes, 6 weeks of growth

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Vigo
Spain

UVIGO (Spain)

Universidade Vigo

Adela Sánchez-Moreiras

Phytotron and Greenhouse

Facility description

- Phytotron and greenhouse for controlled and semi-controlled conditions
- Cereals and legumes
- Never less than 8 replicates
- 2 and 5 L Pots

Specific equipment for root measure

- Scanner EPSON v850 using the Rhizovision software
- Microscopic tools
- Root exudates extraction pump and HPLC and GC-MS tools
- Chlorophyll *a* imaging PAM

Traits measured with specific equipment

- Belowground: specific root length (SRL), specific root surface area (SSA), root diameter, root density, root hairs, number of root tips per root system length, xylem diameter, aerenchyma formation, root exudates.
- Aboveground: ratio of root to leave surface area, leaf length, specific leaf area (SLA), relative water content (RWC), ratio of shoot dry to fresh weight, root:shoot dry mass ratio, chlorophyll *a* fluorescence

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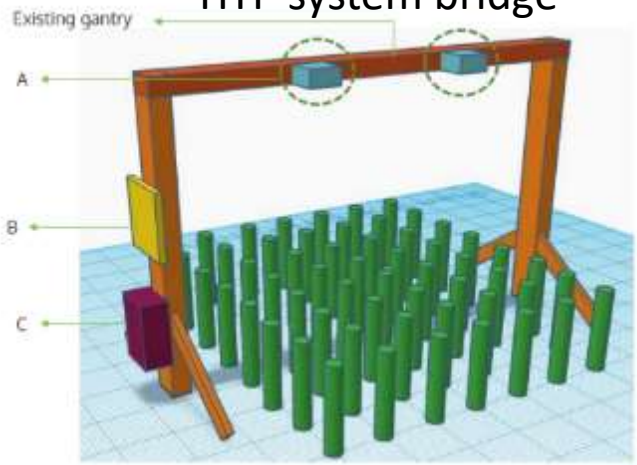
ICARDA (Morocco) PPWP at SEA combines above ground physiology, above ground HTP and below ground traits

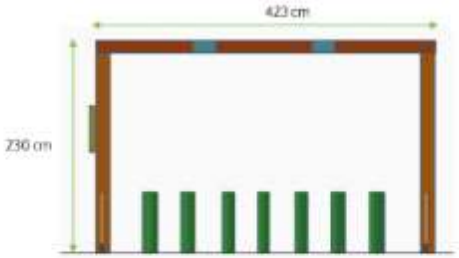
ICARDA
Science for resilient livelihoods in dry areas
CGIAR

Andrea Visioni

Fully Automated Control System
the facility can also be split in different sectors to apply different water regimes/stress levels and or running multiple experiments at the same time

HTP system bridge



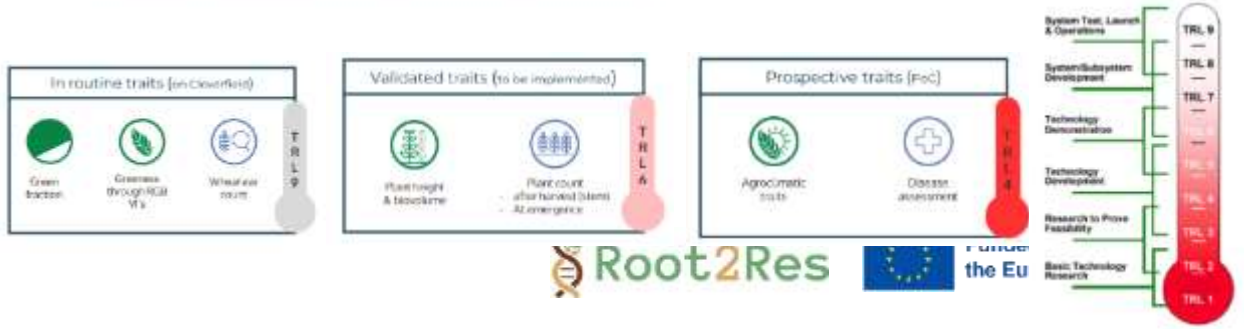


System components:

A – 2 pairs of RGB cameras Sony 24MP (4 cameras)
B – Industrial Tablet
C – Control panel + battery

This system is designed to be easily upgradeable with other sensors (thermal camera, multispectral camera, etc.).

Adult plant root system studies



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Merchuk-Ovnat et al., 2017; https://doi.org/10.3389/fpls.2017.00703

« Phenotyping toolbox» for root architecture traits in controlled conditions

Next steps for controlled environment

- ❑ Clean and share data
- ❑ Compare each facilities
- ❑ Compare root architectural traits with field environment
- ❑ Meta analyses : network, root / aerial part

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Field root phenotyping
Evaluation of different methods

03

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Field capacities for root phenotyping

Shovelomic

ADAS
JHI (excavation)
ARVALIS
KIS
IRTA
ICARDA
ARC

Soil Coring

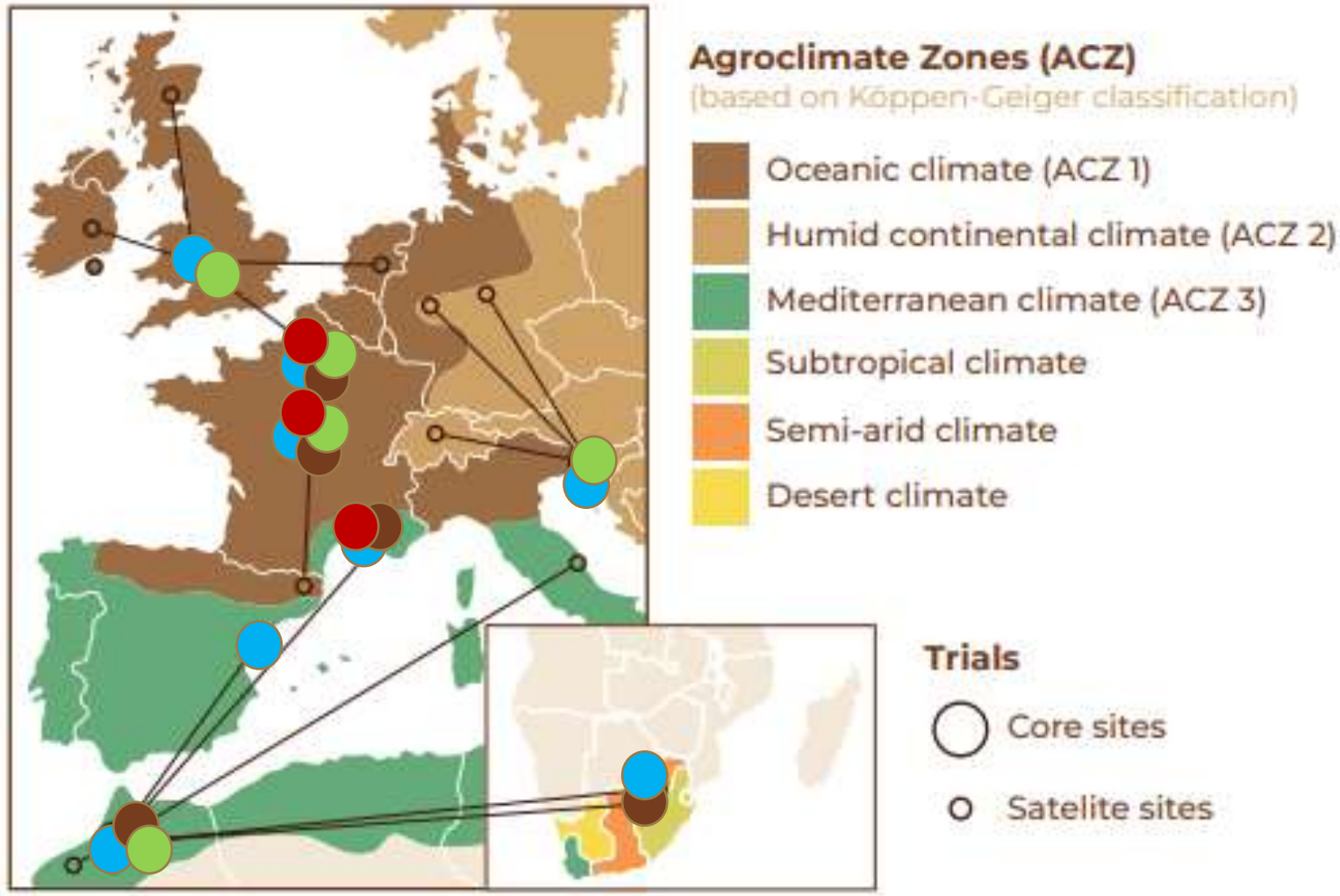
ADAS
ARVALIS
KIS
(ICARDA)

Minirhizotron

ARVALIS
ARC (pot)

Soil pit

ARVALIS



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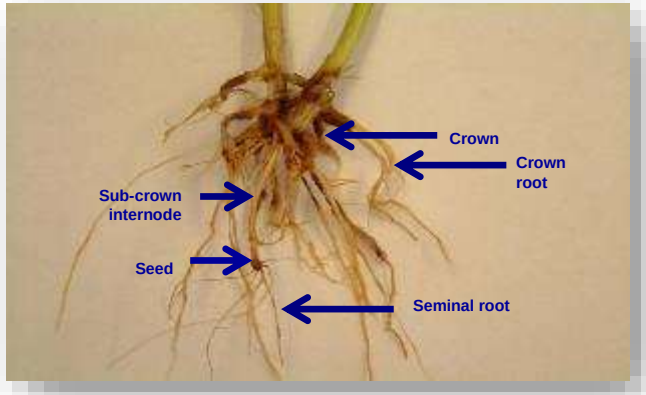
01 Shovelomic

Share and evaluate root phenotyping methods : Shovelomic

Spade sampling
Different architectural traits for each crop

Cereals



Potato

Arvalis shovelomics protocol for potato

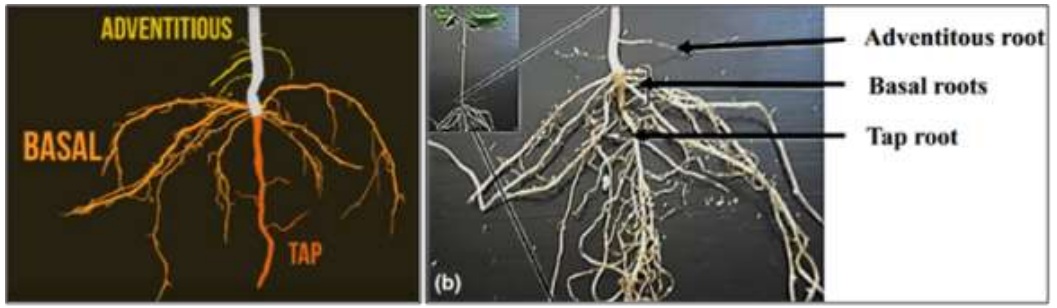
Traits (for 1 plant) :

- Plantlet No
- Stolon No per plant
- Stolon lenghts (for main stem only)
- Basal root No/max lenght per plant
- Total tuber No
- Tuber No > 15mm
- Root scanning with winrhizo

Biomass assessment
(for 4 plants, fresh and dry weights) :

- Stolons
- Underground white stems
- Total root weight
- Mother tubers
- New tubers

Faba bean



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Share and evaluate root phenotyping methods : Shovelomic for potato

Excavation : Extraction of whole potato plants from the field

Time consuming

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Share and evaluate root phenotyping methods : Shovelomic for potato

Easier to carry out the field
Not taking all roots...

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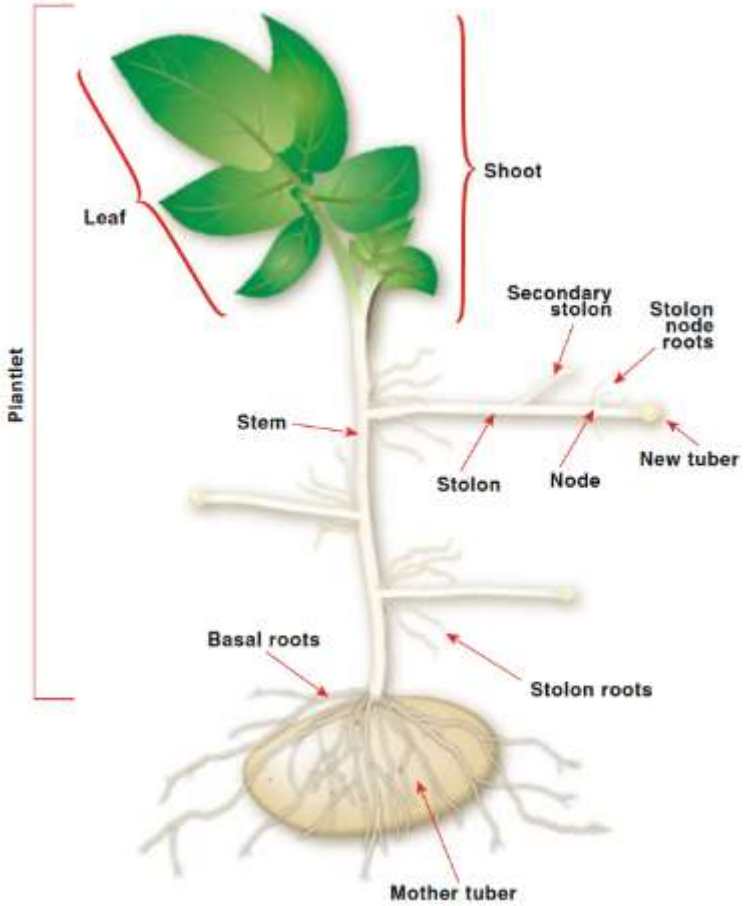
Share and evaluate root phenotyping methods : Shovelomic for potato

Classifying, counting and measuring above and below ground plant



Wishart J et al. 2012

Traits (for 1 plant) :

- Plantlet No per plant
- Stolon No per plant
- Stolon lenghts (for main stem only)
- ~~Stolon roots No/max length per plant~~
- ~~Basal root No/max length per plant~~
- Total tuber No
- Tuber No > 15mm, <15 mm
- Root scanning with winrhizo

Biomass assessment (for 4 plants, dry weights) :

- Underground white stems
- Mother tuber
- New tubers (fresh and dry weight)
- Stolons
- Total root

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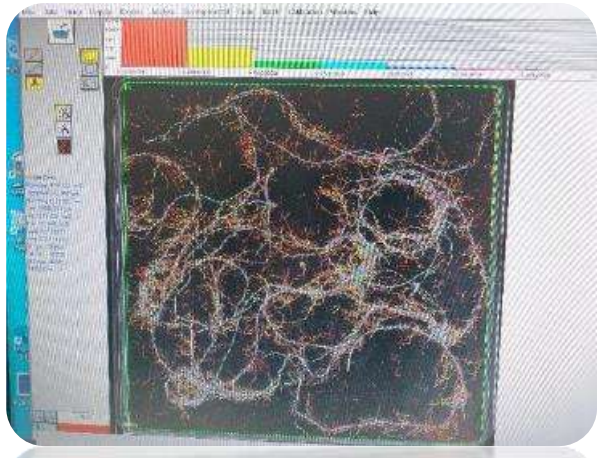
Share and evaluate root phenotyping methods : Shovelomic

Root analysis using WinRHIZO

Total root length, root diameter



Duration

Potato: 1 Sample = 5 plants
Sampling + Cleaning: 2h30
Measure + scanner: 3h15 TOTAL for 5 plants = 6-7h

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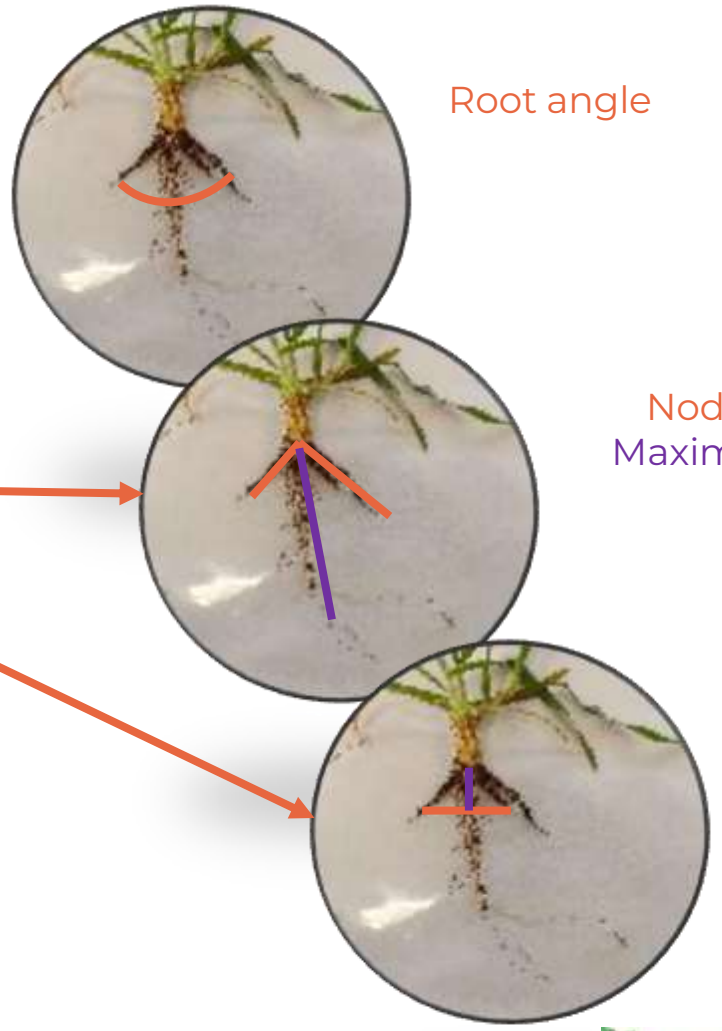
Share and evaluate root phenotyping methods : Shovelomic for cereals

Process

20 cm depth

5-10 plants

Traits per plant



Root angle

Nodal Root length
Maximum root length

Maximum width
Depth to maximum width

Duration

1 Sample = 5 plants Sampling: 7.5 min, Cleaning: 15 min, Measure: 42 min	TOTAL for 5 plants = 1h-1h30

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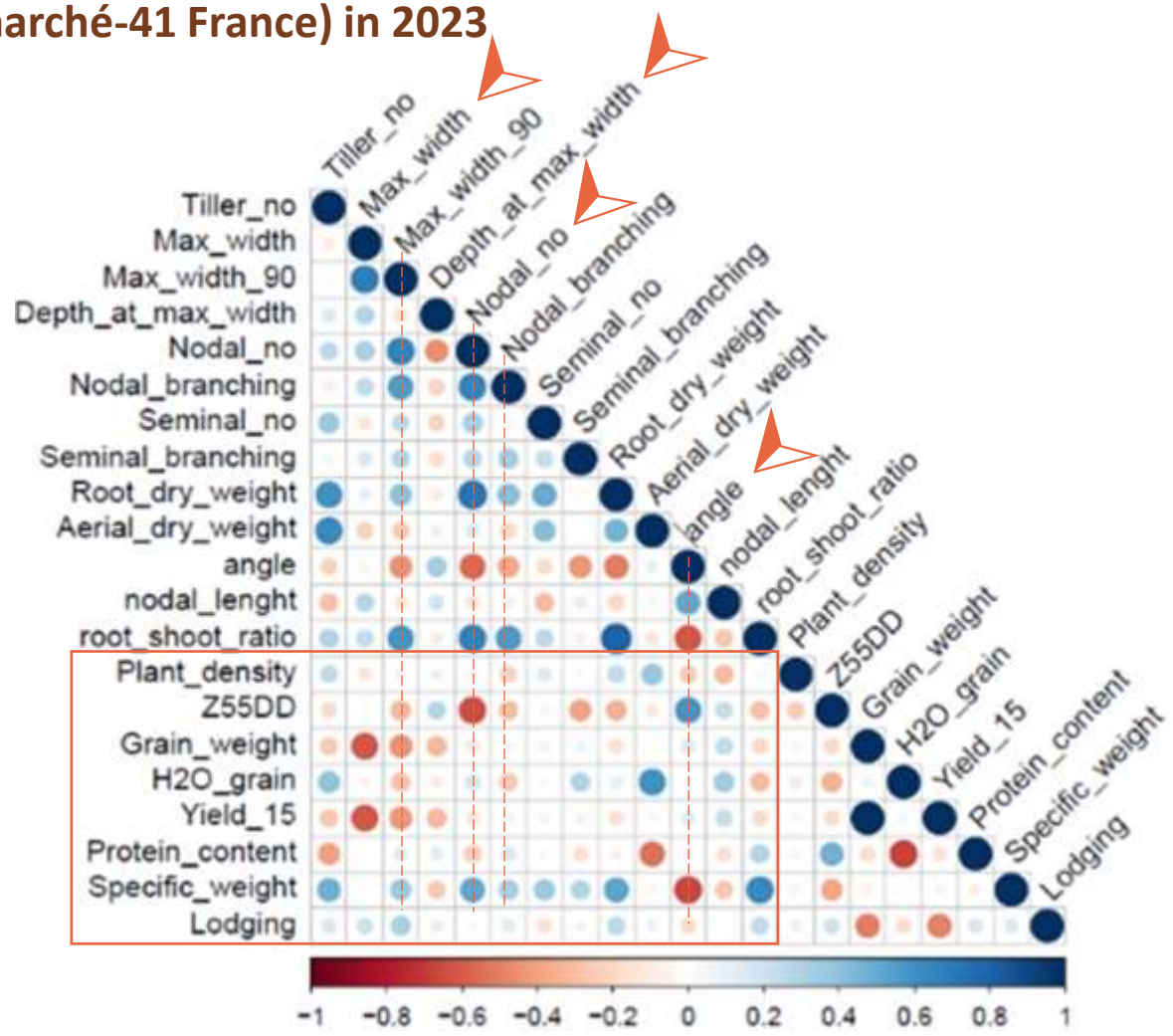
“Phenotyping toolbox” Field methods

Example of results from ARVALIS barley field trial (Ouzouer le marché-41 France) in 2023

Correlation between aboveground data and shovelomic traits.

- Selection of main traits to be measured in genetic panel characterization in 2024.



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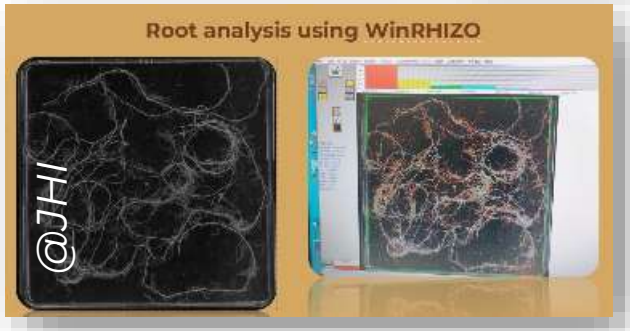
02 Soil coring

Share and evaluate root phenotyping methods : Soil coring

Sample soil volume !



Scanning :

- Total root length
- Root diameter average
- Root length density (cm/cm3)

- Root biomass

Duration

1 sample = 1 tube 140h for cleaning and analysing 24 samples	**TOTAL for 1 tube = 5h50**

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03 Minirhizotron

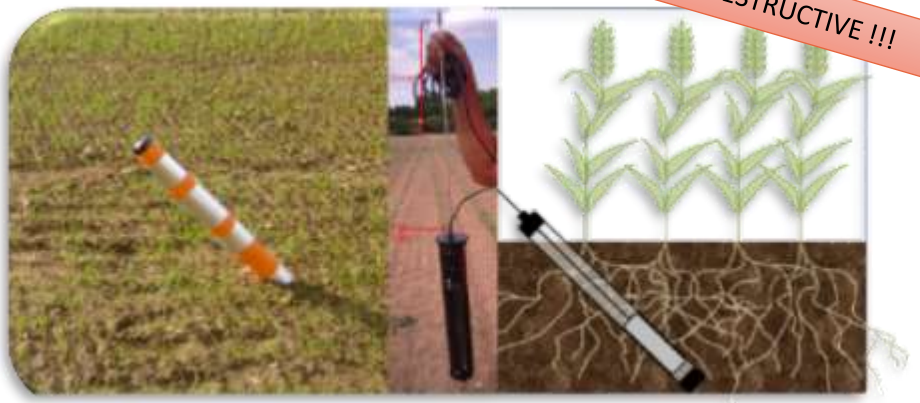
Share and evaluate root phenotyping methods : Minirhizotron

Video sept 2023

Laying tubes

4 tubes/h/pers to put tubes into soil

NON DESTRUCTIVE !!!

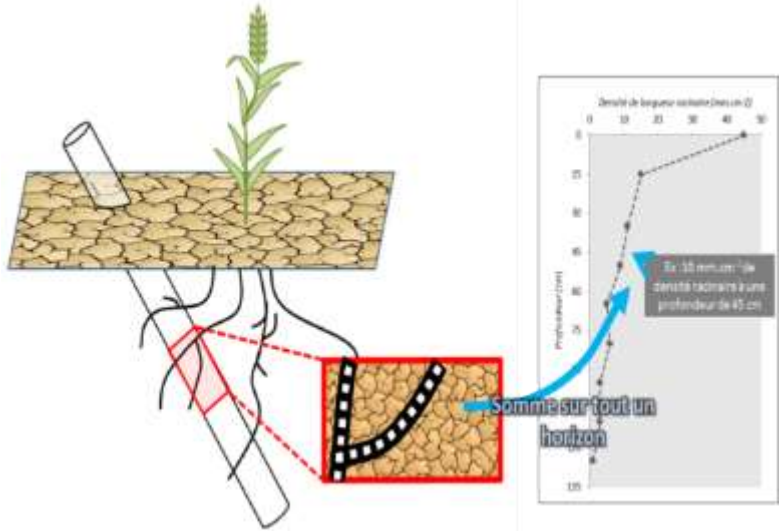


Sampling pictures *(CI-600 de CID Inc., 2017)*

15 min/tubes/pers to sample pictures on each tubes (about 7 per tubes) , Max 30 tubes per day

Image backup and data processing *(ARVALIS Software)*

ARVALIS can process your pictures



'Root length density' = Length of roots present by unit of soil area, by soil depth

'Root diameter' = Average diameter of individual roots, supposed to be cylinders, by soil depth

'Root diameter distribution'= Distribution of average root diameters observed over the entire rooting depth

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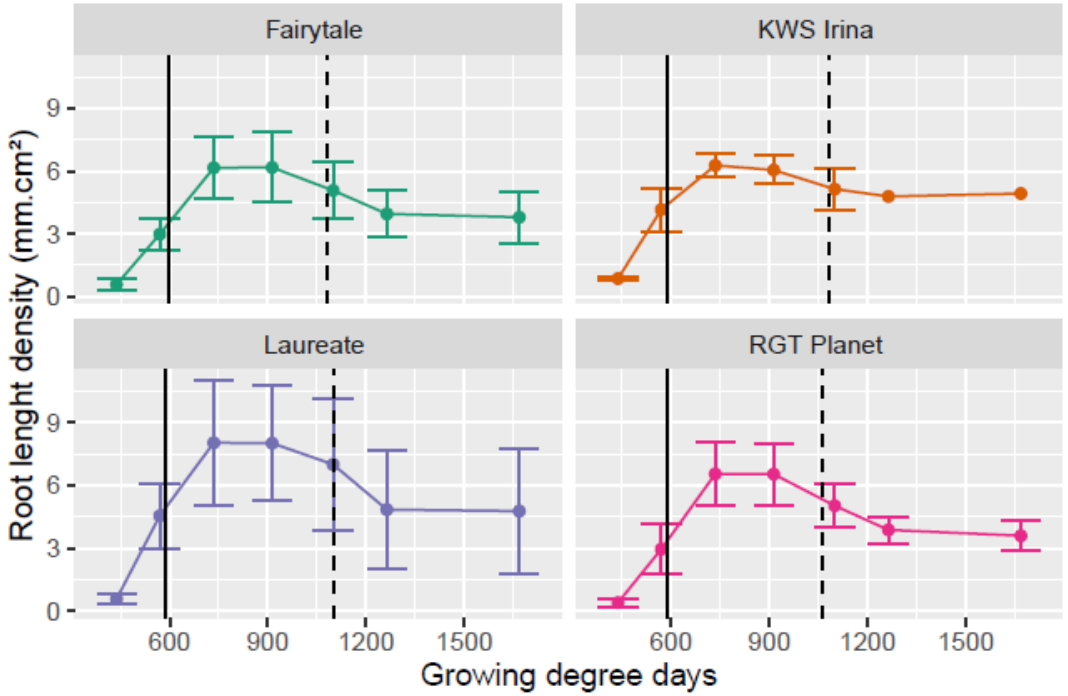
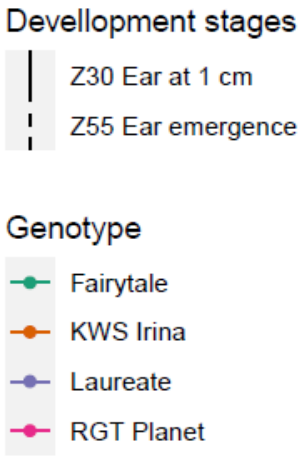
Share and evaluate root phenotyping methods : Minirhizotron

Results from 2023 field barley ARVALIS

Duration

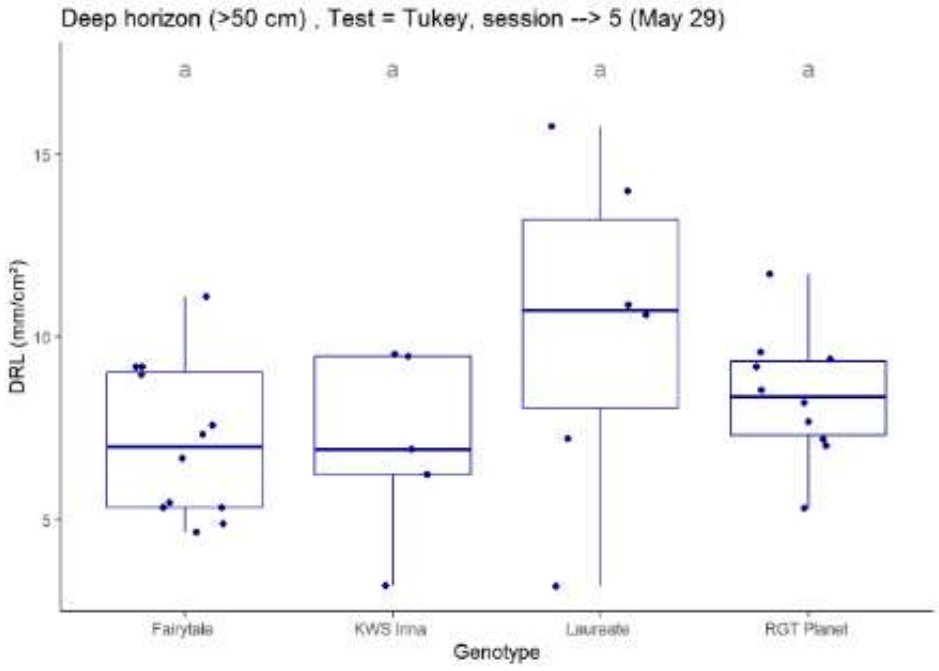
Tube: once a year 15 min / tube /pers Note: 15 min / tube /pers Analyse (software): 1 min all pictures	**TOTAL per tube per date = 20 min**





Trait measured : Root lenght density (mm/cm²)

- High variability
- No differences between varieties



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04 Soil pit

Share and evaluate root phenotyping methods : Soil Pit

ARVALIS-Soil pit-Protocol

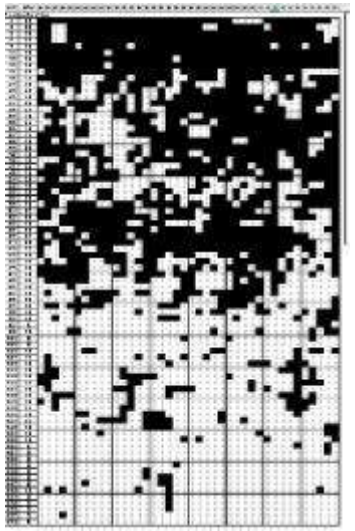
Dig the pit with a backhoe

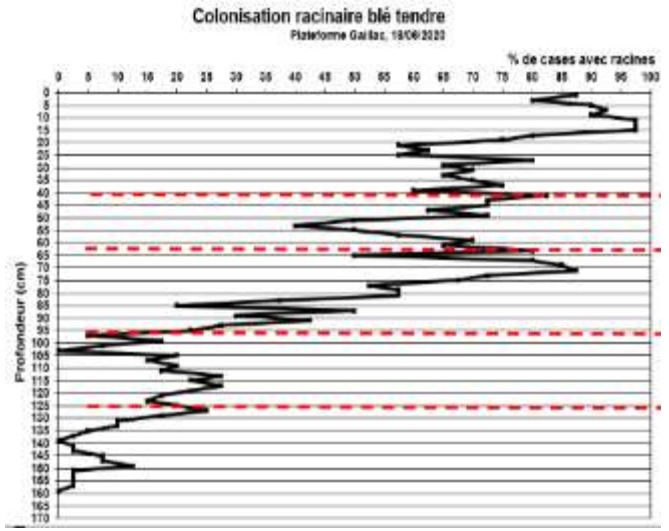
Finish the hole with a shovel and prepare the observation face

Observe the rooting with a grid (e.g. 80*80 cm) with 2 cm square mesh and record it on a sheet

Duration

1 Sample = 80*80 cm 0.64m² Dig the pit : 1h Preparation + Measure : 3h	**TOTAL for 1 grid (1 plot) = 4h**





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05 Results

Results for “Field methods evaluation"

From data available in april 2024

Methods	Cereals Shovelomic	Fababean Shovelomic	Potato Shovelomic	PotatoExcavation	Soil coring	Minirhizotron	Soil Pit
							
Sample	5 plants	5 plants	5 plants	5 plants	850 cm3 per core	600 cm2	10 000 cm2
Depth	0-25 cm	0-25 cm	0-25 cm	0-25 cm	0-80 cm	30-100 cm	0-100 cm
Time per sample	1h30	1h	7 h	39 h	4 h / core	20 min / date	4 h
Cost (invest)	40€ shovel (7 000€ Winrhizo)	40€ shovel (7 000€ Winrhizo)	40€ shovel (7 000 € Winrhizo)	40€ shovel (7 000 € Winrhizo)	36 500 € soil corer 7 000 € Winrhizo	22 000 € rotative scanner	Excavator rental
Main Traits	Root number, angle, lenght, surface area and diameter Root branching index	Root number, angle, lenght, surface area and diameter Root branching index	Root and stolons : biomass, length, surface area and diameter Root branching index	Biomass, length, surface area, diameter and root branching index for stolons, stolon roots, node roots de stolons and mother tuber roots	Root biomass, length, surface area and diameter	Dynamic root length density (cm.cm²) and dynamic avreage of root diameter	Root density (presence/absence of roots per cm²)
Disadvantages	First horizon sampling	First horizon sampling	First horizon sampling	Extremely time consuming	Time consuming	Not available on the first horizon (0-30 cm)	Very destructive, once
Advantages	Easy, quick	Easy, quick	Easy to apply 3D architecture, main biomass	Comprehensive measurement of all roots in the ridge	Deep rooting, root biomass all over 80 cm depth	Non destructive ! Dynamic measurements on deep rooting	Wide and deep field of view

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Results for “Field methods evaluation"

From data available in april 2024

Methods	Cereals Shovelomic	Soil coring	Minirhizotron	Soil Pit
				
Sample	5 plants	850 cm3 per core	600 cm2	10 000 cm2
Depth	0-25 cm	0-80 cm	30-100 cm	0-100 cm
Time per sample	1h30	4 h / core	20 min / date	4 h
Cost (invest)	40€ shovel (7 000€ Winrhizo)	36 500 € soil corer 7 000 € Winrhizo	22 000 € rotative scanner	Excavator rental
Main Traits	Root number, angle, lenght, surface area and diameter Root branching index	Root biomass, length, surface area and diameter	Dynamic root length density (cm.cm²) and dynamic avreage of root diameter	Root density (presence/absence of roots per cm²)
Disadvantages	First horizon sampling	Time consuming	Not available on the first horizon (0-30 cm)	Very destructive, once
Advantages	Easy, quick	Deep rooting, root biomass all over 80 cm depth	Non destructive ! Dynamic measurements on deep rooting	Wide and deep field of view

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Field methods to identify and validate ideotype traits

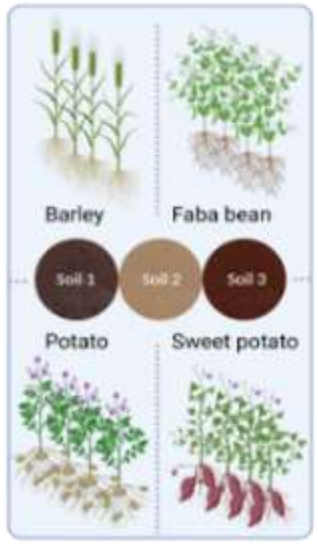
	Traits	Shovelomic	Soil coring	Minirhizotron	Soil pit
Root Traits – Architectural/developmental	**Greater root dry weight**	✓ for 0-20 cm depth	✓ for 0-80 cm depth	-	-
	Deeper rooting	- *Root angle ?*	✓	✓	✓
	Increased lateral root number	✓	-	-	-
	Early root vigour	✓	-	✓	-
	Increased distribution of roots at depth	-	✓	✓	✓
	Root surface area	✓ *Possible for 0-20 cm depth*	✓	- *(dev possible)*	-
	Increased root length density	-	✓ cm/cm3	✓ mm/**cm2**	-

ROOT2RES “Phenotyping toolbox” Deliverables

Standard Operating Procedure, video and practice abstracts

- Shovelomic
- Soil coring
- Minirhizotron
- Soil pit

D2.1 Phenotyping toolbox-architectural traits
August 2023



D2.3 Phenotyping toolbox-rhizosphere
September 2023

D2.2 Phenotyping workshop
Febuary 2024

ACZ1: 29/06/23 Northern France, Field potato
ACZ2: 05-06/02/24 Julich Germany, CE
ACZ3 : 29/02/24 Morocco, Field cereals

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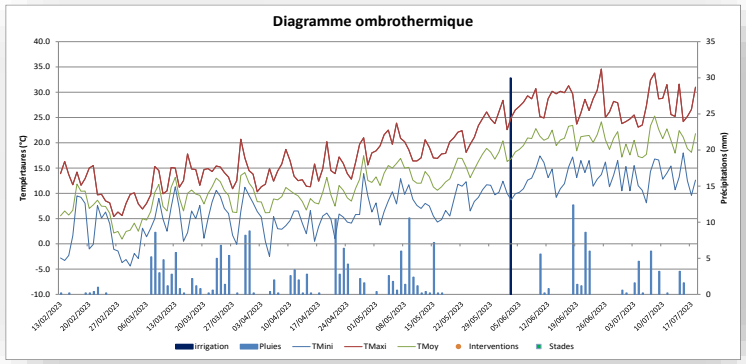
Root2Res Mid term conference

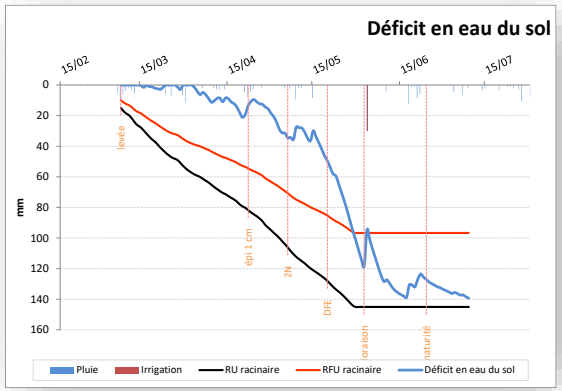
Next steps
Update SOP for breeding

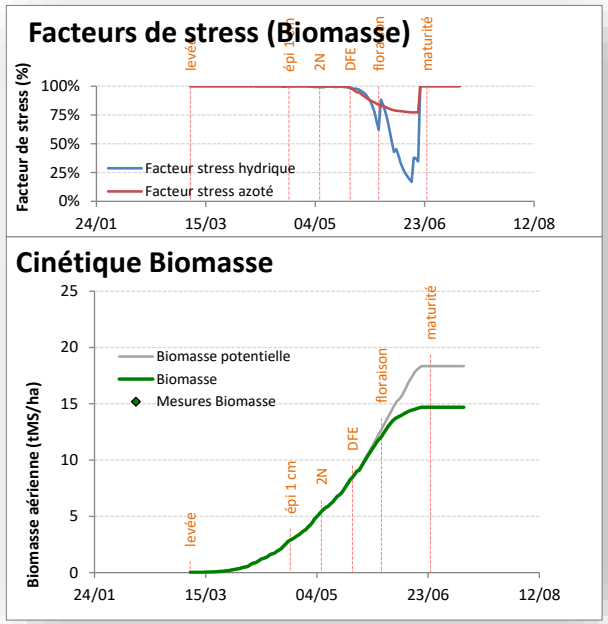
04

Envirotyping

- Specific meeting with soil specialist (WP2 site and WP5) => 24/02/23 to define data needed for envirotyping work **ProtocolTraits.xlsx**
- Collecting data from ARVALIS
  Still on going, **difficult to have soil data from each location before or during the trial**
- Meetings on CHN and irriguide models and input to run them







Next steps

- Collect soil data and weather data from each trial site
- Choose envitotyping traits
- Take these input to run envirotyping models
- Classify each trial

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Root phenotyping methods : next steps

- Compare root traits measured with different methods at the network level : in field, in controlled conditions and both !
- Compare root growth and above ground development
- Search yield correlation with a root system characterization
- Update field methods to be able to apply new version of standard operating procedure on “big” panel

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Thanks

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Funded by the European Union

UK Research and Innovation

Project funded by

Swiss Confederation

Federal Department of Economic Affairs, Education and Research EAER
State Secretariat for Education, Research and Innovation SERI