UNIVERSITY OF SÃO PAULO

LGN5831 - SPECIAL TOPICS IN GENETICS AND PLANT BREEDING

Shovelomics

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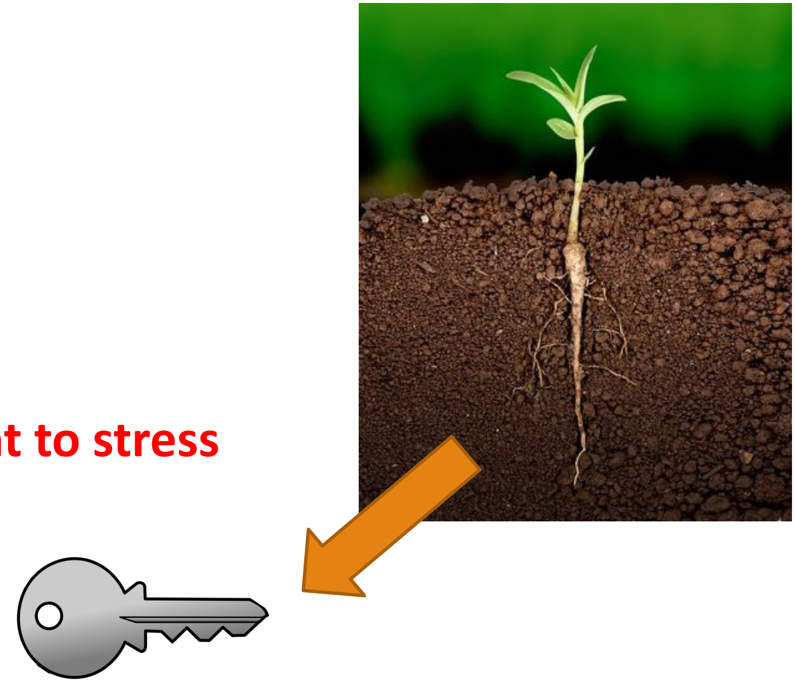
Introduction

Root System

- Major organ system of the plant body
- Functions:
  - Anchorage and support
  - Absorption (nutrition)
  - Storage
  - Reproduction
  - **Metabolic reactions – adjustment to stress**



Concept

Shovelomics

High throughput phenotyping root architecture in the field

Trachsel et al. (2011)

- Visual scoring of excavated root crowns to assess different root architecture traits
- Field-based
- Root excavation
- Manual phenotyping
- Simple and robust

Root characterization

- **Morphology**: surface features, characteristics of the epidermis, root diameter etc.;
- **Topology**: branching;
- **Distribution**: presence of roots in a positional gradient or grid;
- **Architecture (RSA):** Spatial configuration (geometric deployment of root axes).

Root characterization

RSA

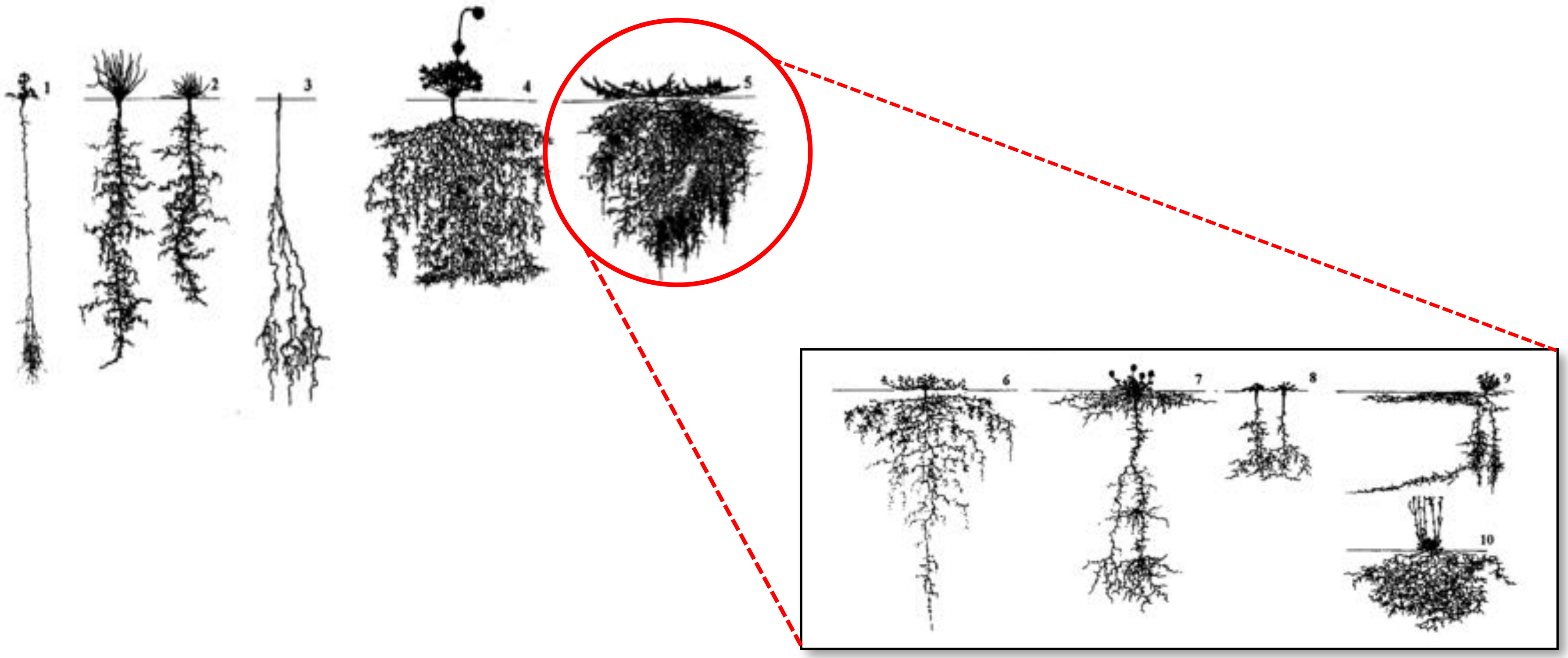
- Important for soil resource acquisition
- Better understanding of genetic, physiological, and environmental regulation

Traits

- Root elongation
- Growth angles
- Lateral branching

Root characterization

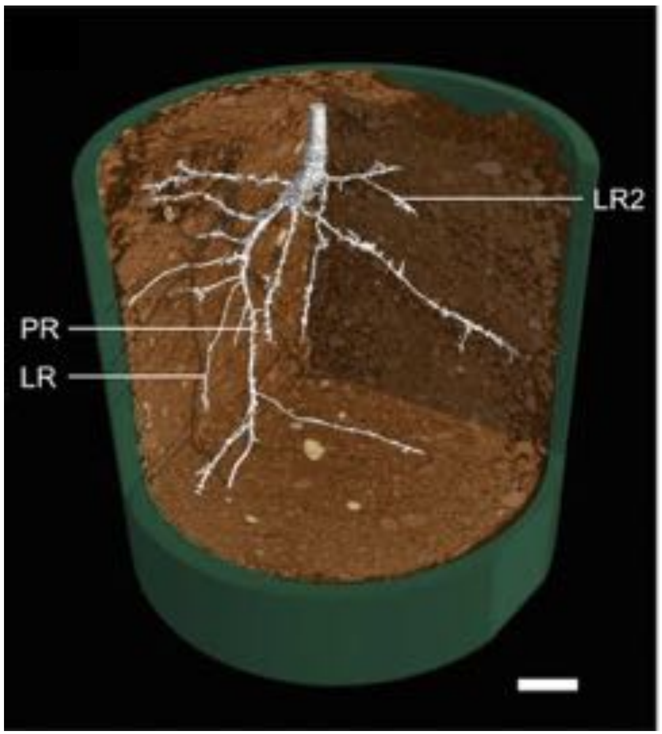
Inter



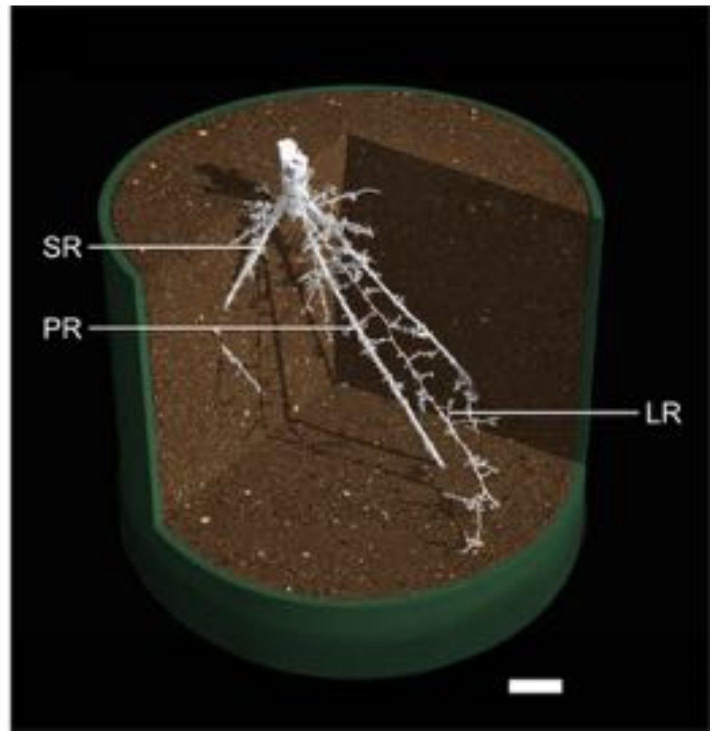
Intra

Root characterization

Tomato



Wheat



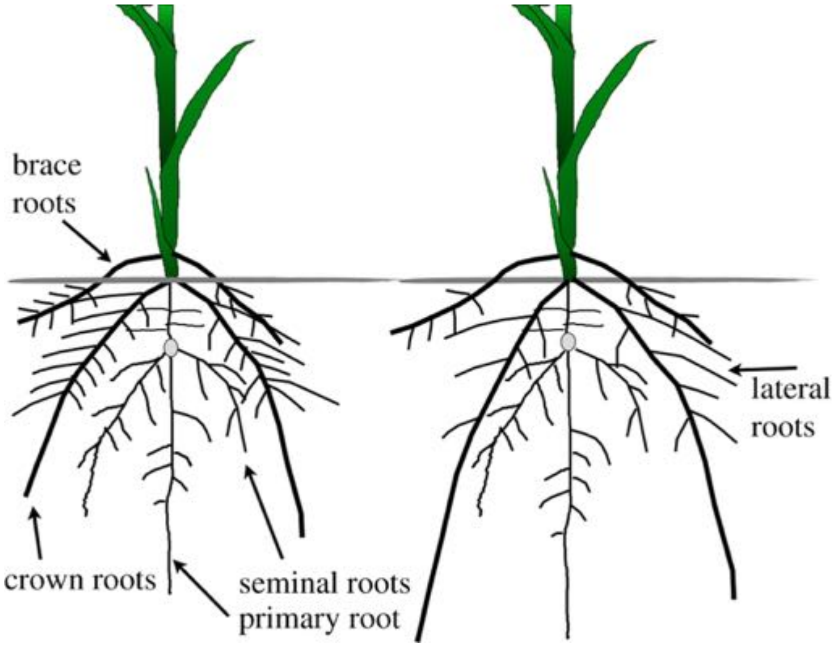
Importance

Wide range of areas:

- Physiology
- Pests and radicular diseases
- **Plant breeding**
  - **Abiotic stresses**
    - **Nutrients**
    - **Water**

Root x Stresses

Root system is greatly impacted by abiotic stresses



Better resources use efficiency:

- Different RSA
- Different capacities of soil exploration

Plant breeding context

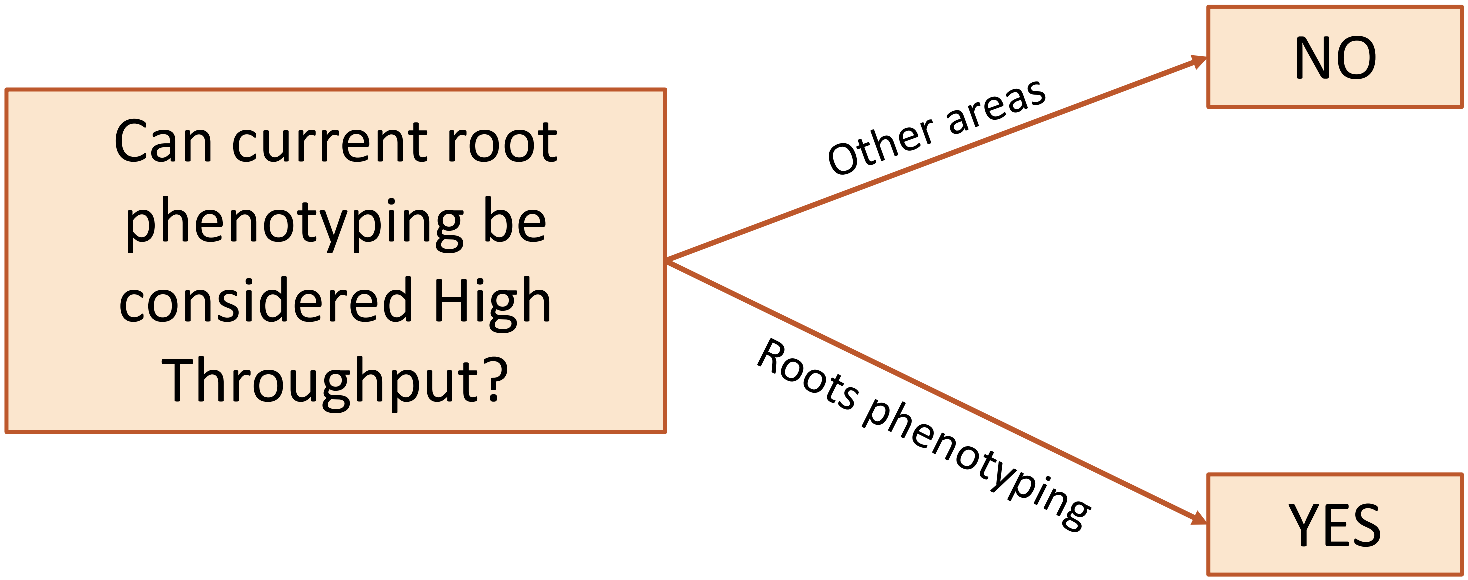
- Genotypic variation for soil resource acquisition may be largely untapped in crop breeding programs
  - Focused on adaptation to high-input systems
  - Root traits as selection criteria

Shovelomics

Challenges

- High heterogeneity in the soil
- Sampling a large number of plants
- Field evaluations
- Cost
- Destructive analysis
- Damage to root system
- Time consuming

HTP?


Can current root phenotyping be considered High Throughput?
Other areas
NO
Roots phenotyping
YES

Strategies

Components

Two main components concerning root phenotyping:

- Method for culturing the plants

Laboratory
- Controlled growth
- No physiology evaluation

Greenhouse
- Intermediate
- No competition evaluation

Field
- Physiological relevance
- Labor and time intensive

- Analysis tool

Strategies

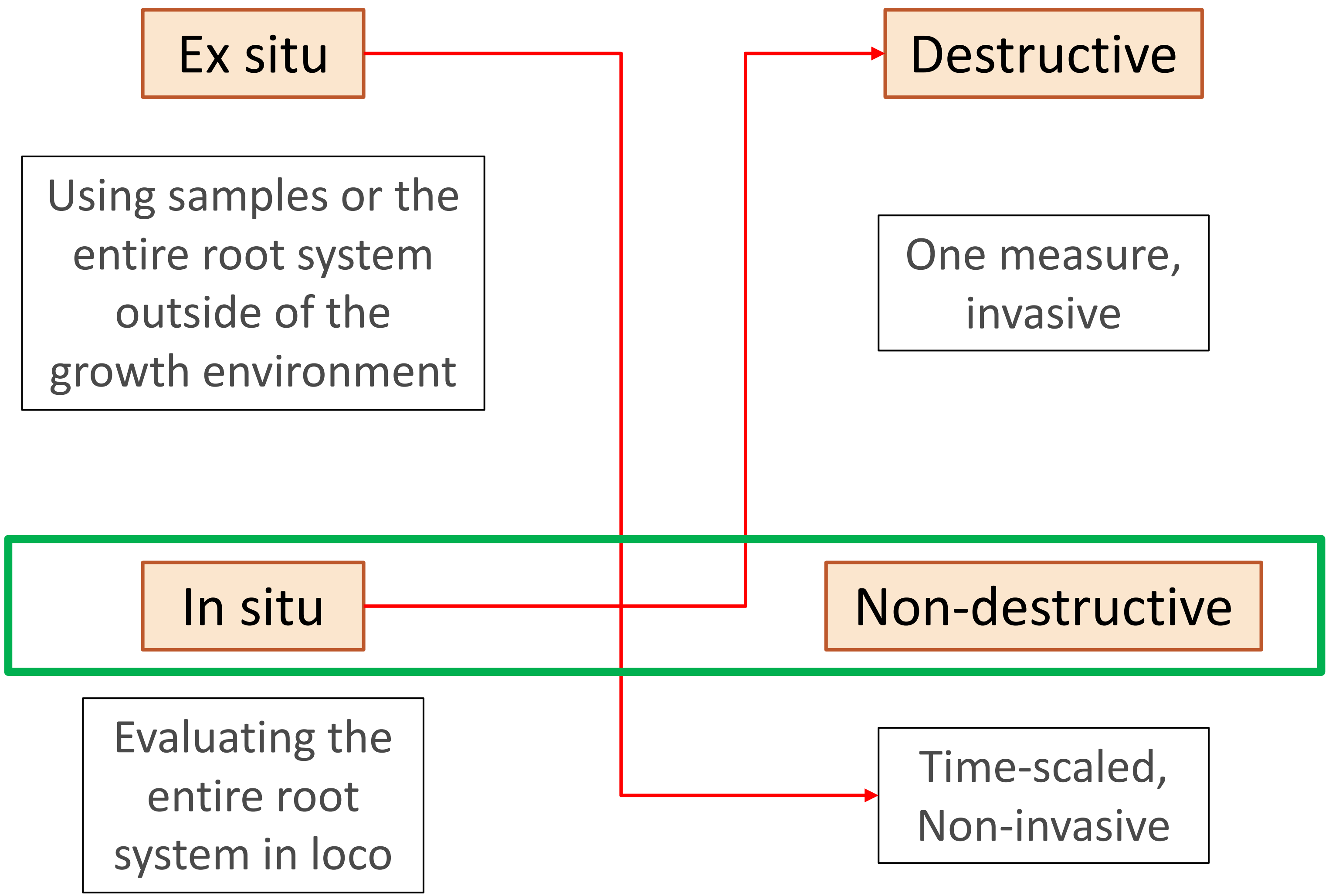
They must be aligned to the research goal

- Objective:

answer basic root developmental questions	high throughput root trait selection for breeding

- Specific root trait of interest
- Desired time-scaling for sampling
- Infrastructure capacity
- Cost viability

Classification



Strategies

Plant Cultivation System	Growth Media	Description
1. Growth and luminescence observatory for roots (GLO-Roots)	Soil (lab)	This method combines custom-made growth vessels and new image analysis algorithms to non-destructively monitor RSA development over space (2-D) and time. The technique allows information on soil properties (e.g., moisture) to be integrated with root growth data. The system makes use of luminescence imaging of roots expressing plant codon-optimize luciferase.
2. X-Ray computed tomography	Soil (lab and greenhouse)	Non-destructively visualizes opaque root structures by measuring the attenuation of ionizing radiation as it passes through the root. A series of projections are acquired and combined to reconstruct a 3D image of the root system.
3. Rhizophonics	Liquid media (lab)	Combines hydroponics and rhizotrons. System is made of a nylon fabric supported by an aluminum frame. The set-up is immersed in a tank filled with liquid media. Allows non-destructive, 2-D imaging of root architecture while simultaneously sampling shoots.
4. Clear pot method	Soil (greenhouse)	Uses transparent pots filled with soil or other potting media. Seeds are planted close to the pot wall to enable high- throughput imaging of roots along the clear pot wall. To prevent light exposure, the clear pot is placed in black pots while roots are developing.
5. Rhizoslides	Paper-based (lab, greenhouse)	The set-up consists of a plexiglass sheet covered with moistened germination paper. Seeds are planted on the slit of the plexiglass. The system allows separation of crown roots from embryonic roots.
6. Shovelomics	Soil (field-based)	Involves manual excavation of plants and separating roots from the shoots. Washed roots are then placed on a phenotyping board for root trait quantification. New algorithms allow extraction of several root traits in a high throughput manner.
7. Soil coring	Soil (field-based)	Uses a tractor-mounted, hydraulic soil corer to drive steel alloy sampling tubes into the soil. When combined with novel planting configurations (e.g., hill plots), this method allows for phenotyping deep rooted crop varieties.
8. Rhizolysimeters	Soil (field-based)	Elaborate facility consisting of an underground corridor and concrete silos and pipes to house soil-containing soil cores for direct root observation.
9. Minirhizotrons	Soil (field-based)	A transparent observation tube permanently inserted in the soil. Images of roots growing along the minirhizotron wall at particular locations in the soil profile can be captured over time.

Strategies

RootReader3D	RootReader3D software is designed to reconstruct and quantify 3D root system architecture descriptors from 2D rotational image sequences	Root length, diameter and surface area; root depth and volume; convex hull; number of branches; root orientation; insertion angles	OD	Automated	Seedlings to mature plants	Projection, any	Clark et al. (2011)	www.plantmineralnutrition.net/rootreader.htm
RootScape	RootScape is a landmark-based allometric method for rapid phenotyping of root system architecture	Root shape	F	Semiautomated	Arabidopsis	gif, tiff, jpg, png	Ristova et al. (2013)	www.aimsnstudio.com/Rootscape
RootSnap!	The software has tracing enhancements to snap root tracing points to the centre of the root automatically. It can monitor root growth, disease, dynamics and behaviour over time and simplify mapping roots	Root length, diameter, surface area and volume	C	Semiautomated	Any	Any	Juraniec et al. (2014)	www.cid-inc.com/root-snap
RootTrace	RootTrace allows automatic and high-throughput measure of root length and curvature. It can trace the main root to the tip in every image in a time series. The software has been extended to count emerged lateral roots and to recover strongly curved and agravitropic roots	Root length; curvature; number of branches	O&F	Automated	Arabidopsis, seedlings	Time series	French et al. (2009)	sourceforge.net/projects/roottrace/

Ex-situ pioneers

Hydroponics, culture medium, pots

Hydroponics, culture medium, pots

Pros

Lower cost

Controlled conditions

Easy characterization

High correlation

Cons

Root volume limited

Restrict root growth

Destructive

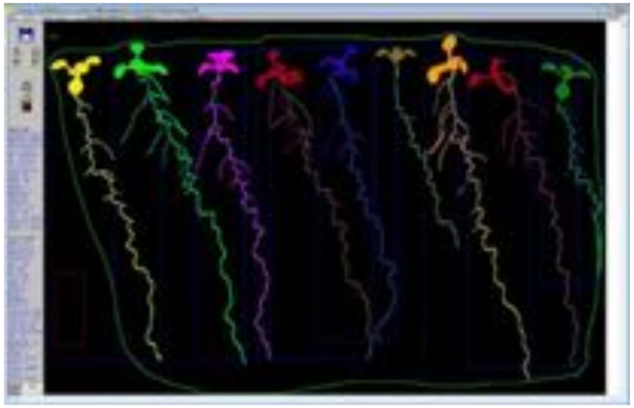
Time consuming

Do not represent natural soil conditions

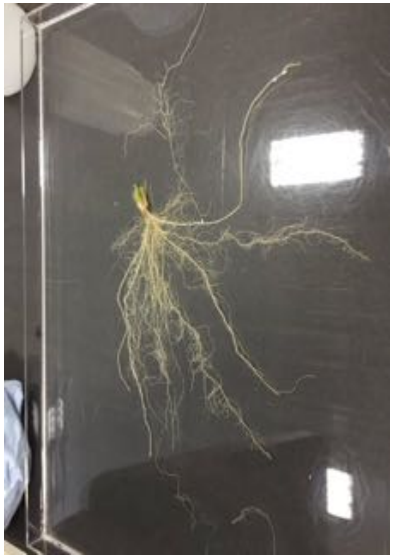
WinRHIZO

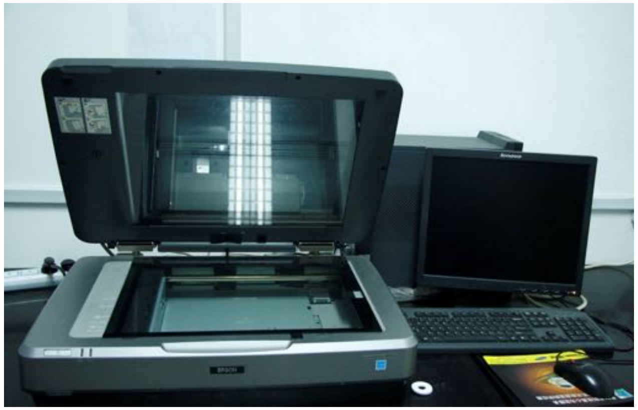
WinRHIZO

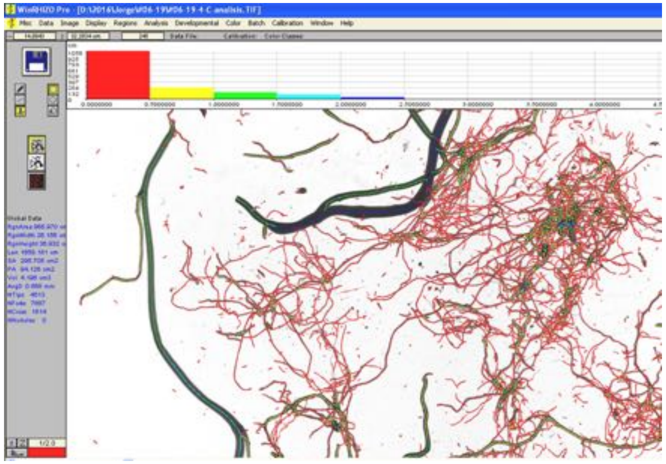
- Scanning combining with computerized image analysis
- Image analysis system specifically designed for root measurement in different forms
- Made of a computer program and image acquisition components
- Information: morphology (length, area, volume...), topology, architecture and color analyses.



Procedure







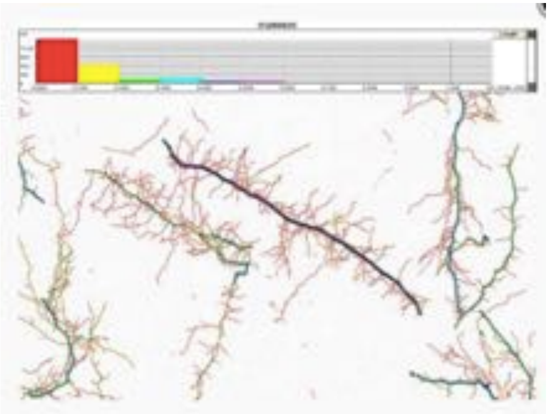
Measures

- As a function of root diameter or color, distribution graphic displays:
    - root length
    - area
    - volume
    - number of tips

	A	B	C	D	E	F	G	H	I	J
1	General Information About The Analysis									
2	RHIZO 2002c	Operator	Date	Time	ImgType-PxSizeH-PxSizeV	AnalysedRgn	Left-Top-Right-Bottom	NExclusions	CalibMethod	PxClassif
3	SAMPLE NAME	YOUR NAME	9/26/06	10:25:41	Grey-0.01264	Rgn	4-168-1060-	0	Intrinsic	GreyThdMan
4	Length, Area, and Diameter Data									
5	Filters	Fractal-PxMin-Max	Length(cm)	ProjArea(cm2)	SurfArea(cm2)	AvgDiam(mm)	RootVolume (cm3)	Tips	Forks	Crossings
6	SmoothOffAn	FractalOff	333.5113	14.1542	44.4667	0.4244	0.472	169	631	188
8	Length by Diameter Class									
9	0.00<.L.<=0.25	0.25<.L.<=0.50	0.50<.L.<=0.75	0.75<.L.<=1.00	1.00<.L.<=1.25	1.25<.L.<=1.50	1.50<.L.<=1.75	1.75<.L.<=2.00	2.00<.L.<=2.25	.L.>2.25
10	76.3316	177.4507	29.7995	29.4336	15.0368	3.6591	0.6429	0.3219	0.4482	0.3696
11	Surface Area by Diameter Class									
12	0.00<.SA.<=0.25	0.25<.SA.<=0.50	0.50<.SA.<=0.75	0.75<.SA.<=1.00	1.00<.SA.<=1.25	1.25<.SA.<=1.50	1.50<.SA.<=1.75	1.75<.SA.<=2.00	2.00<.SA.<=2.25	.SA.>2.25
13	2.9924	18.6135	4.8859	7.9181	4.8976	1.4938	0.3273	0.182	0.2924	0.2819
14	Projected Area by Diameter Class									
15	0.00<.PA.<=0.25	0.25<.PA.<=0.50	0.50<.PA.<=0.75	0.75<.PA.<=1.00	1.00<.PA.<=1.25	1.25<.PA.<=1.50	1.50<.PA.<=1.75	1.75<.PA.<=2.00	2.00<.PA.<=2.25	.PA.>2.25
16	0.9525	5.9249	1.5552	2.5204	1.559	0.4755	0.1042	0.0579	0.0931	0.0897
17	Root Volume by Diameter Class									
18	0.00<.V.<=0.25	0.25<.V.<=0.50	0.50<.V.<=0.75	0.75<.V.<=1.00	1.00<.V.<=1.25	1.25<.V.<=1.50	1.50<.V.<=1.75	1.75<.V.<=2.00	2.00<.V.<=2.25	.V.>2.25
19	0.0095	0.1606	0.0641	0.1702	0.1272	0.0486	0.0133	0.0082	0.0152	0.0172
20	Number of Tips by Diameter Class									
21	0.00<.T.<=0.25	0.25<.T.<=0.50	0.50<.T.<=0.75	0.75<.T.<=1.00	1.00<.T.<=1.25	1.25<.T.<=1.50	1.50<.T.<=1.75	1.75<.T.<=2.00	2.00<.T.<=2.25	.T.>2.25
22	111	62	12	4	0	0	0	0	0	0

- Number and the width of the classes: user-definable

- The color used to draw the root skeleton indicates into which diameter class the part of the root has been classified.



- Measurement data of the sample under analysis is summarized on screen and is available in detail in data files.

WinRHIZO

Pros

Many measures

Precision by colors and configurations

Easy data obtention

Rapid estimates

Cons

Destructive

Limit of read (stages)

Mathematical disadvantage

Washed roots: may be a problem

Slowness

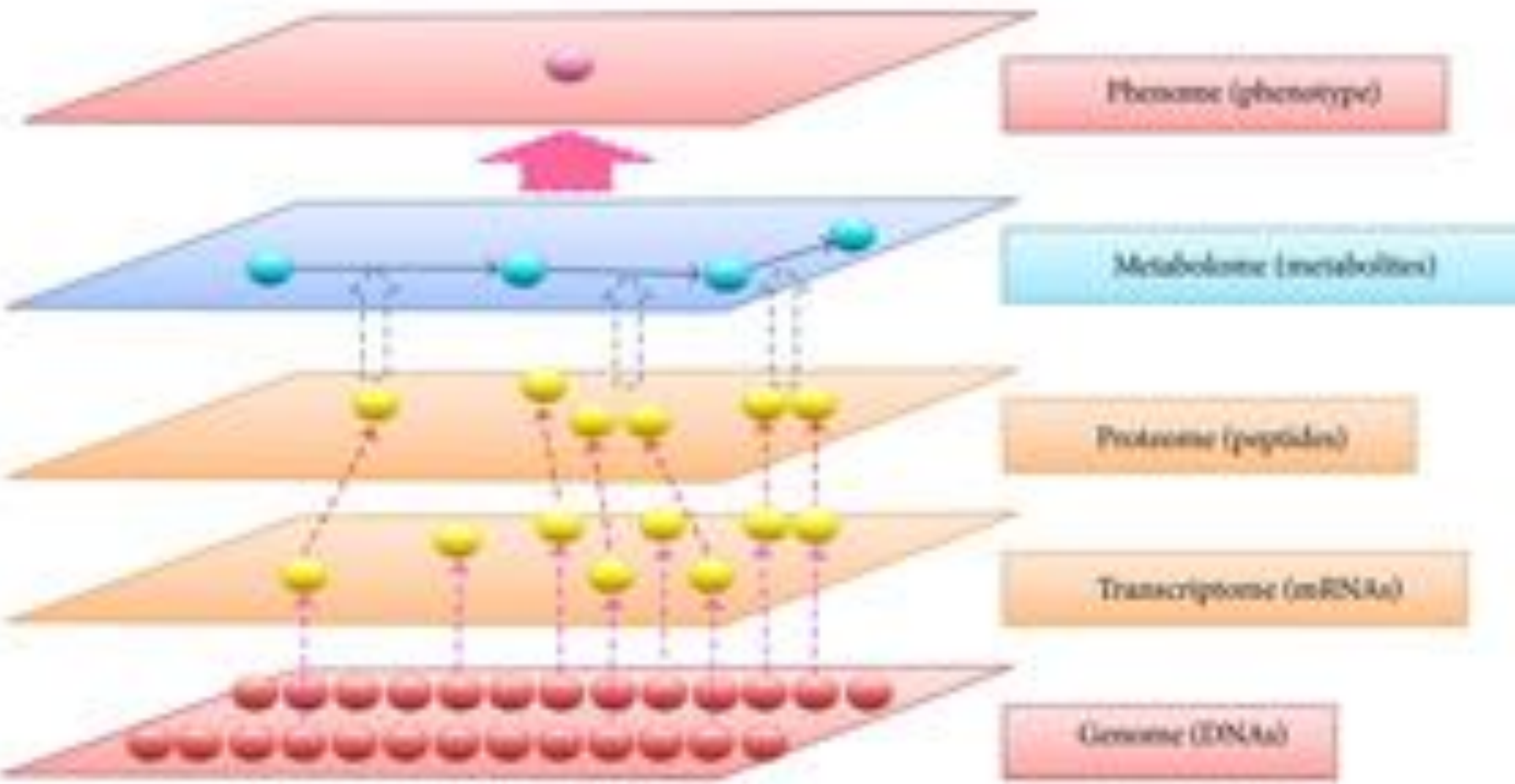
Information from RSA is lost

GLO-Root system

GLO-Root system

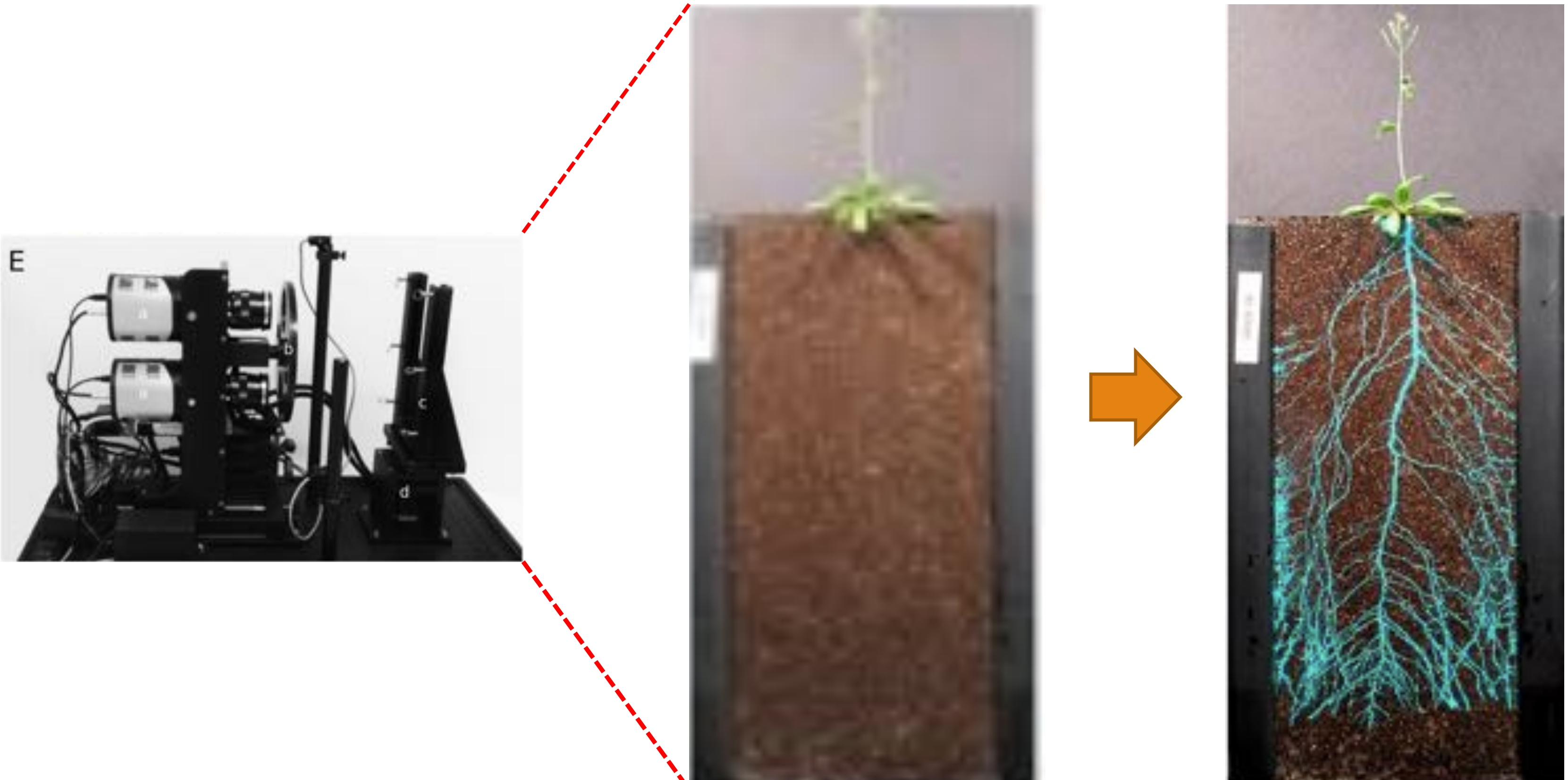
- Growth and Luminescence Observatory for Roots
- Integrated platform for growing plants in soil
  - in custom-built vessels,
  - imaging roots using bioluminescence
  - analyzing root growth, architecture and gene expression
- Genetic engineering of plants to produce luciferase, which causes them to glow in the dark of the soil
- Simultaneously tracks whole root system architecture and the gene expression of adult plants

GLO-Root system

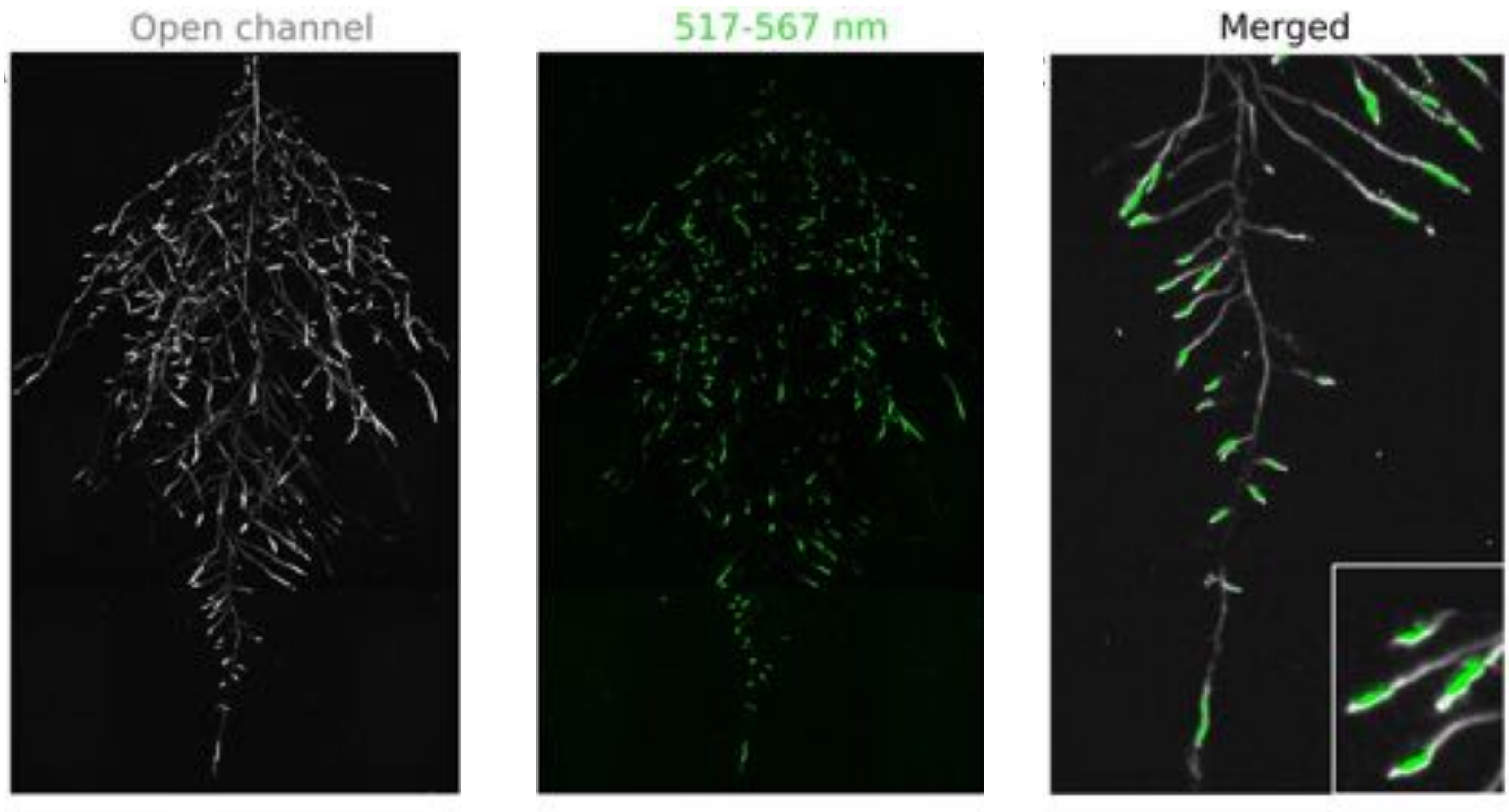


GLO-Root system

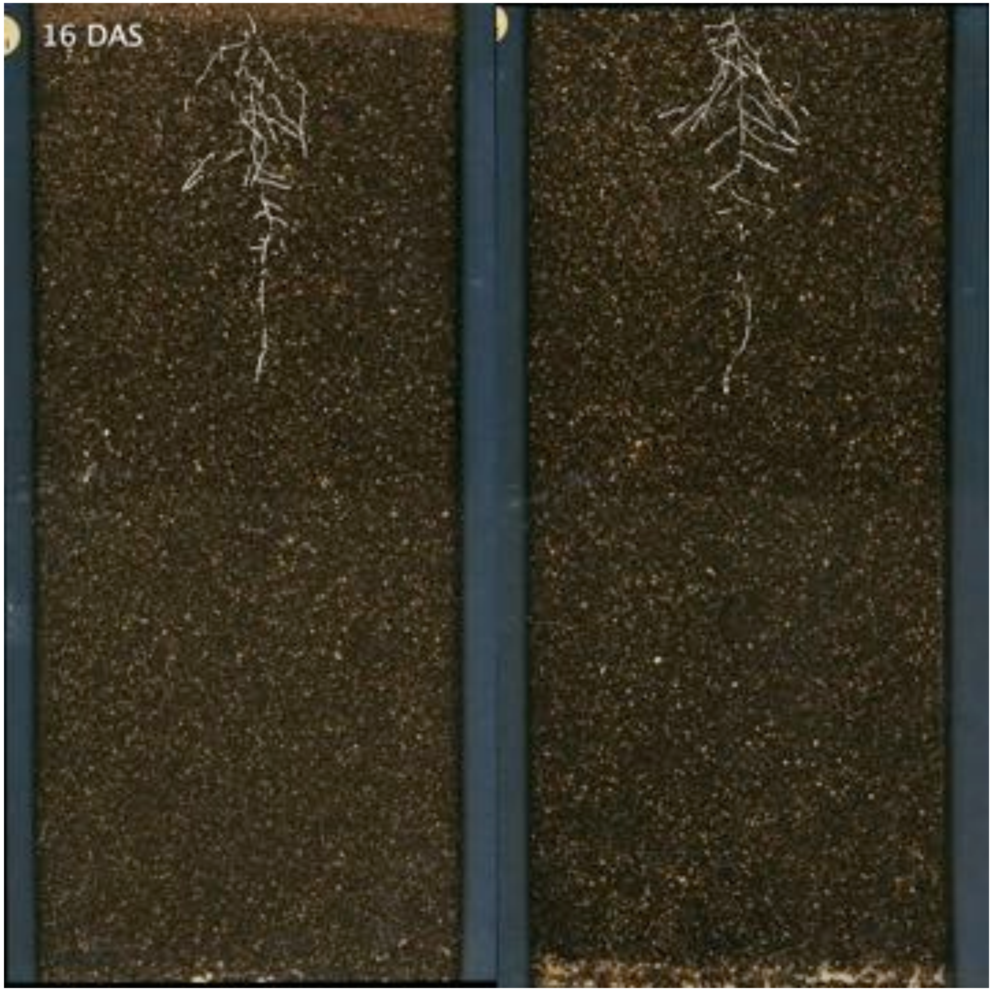
GLO-Roots system



GLO-Root system



In this perspective…

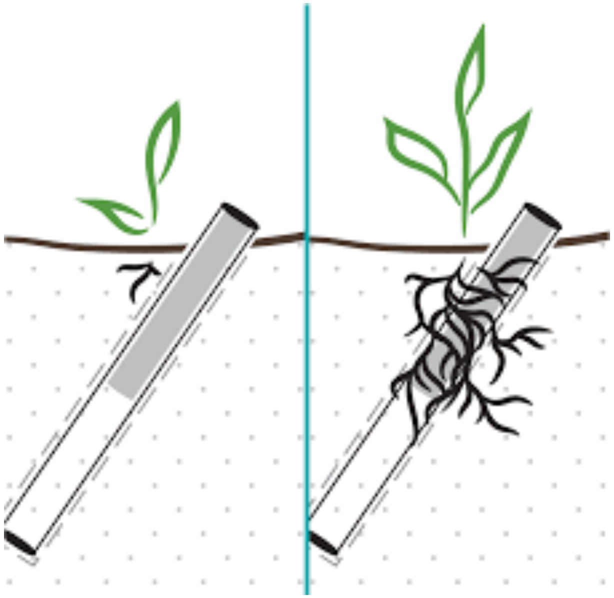


CI-600 Root Imager

CI-600 Root Imager

- Minirhizotron - ability to capture non-destructive, high-resolution, digital images of living roots in soil over multiple growing seasons.

- Free root analysis software: **RootSnap!**
  - root length,
  - area,
  - volume, diameter,
  - branching angle



CI-600 Root Imager

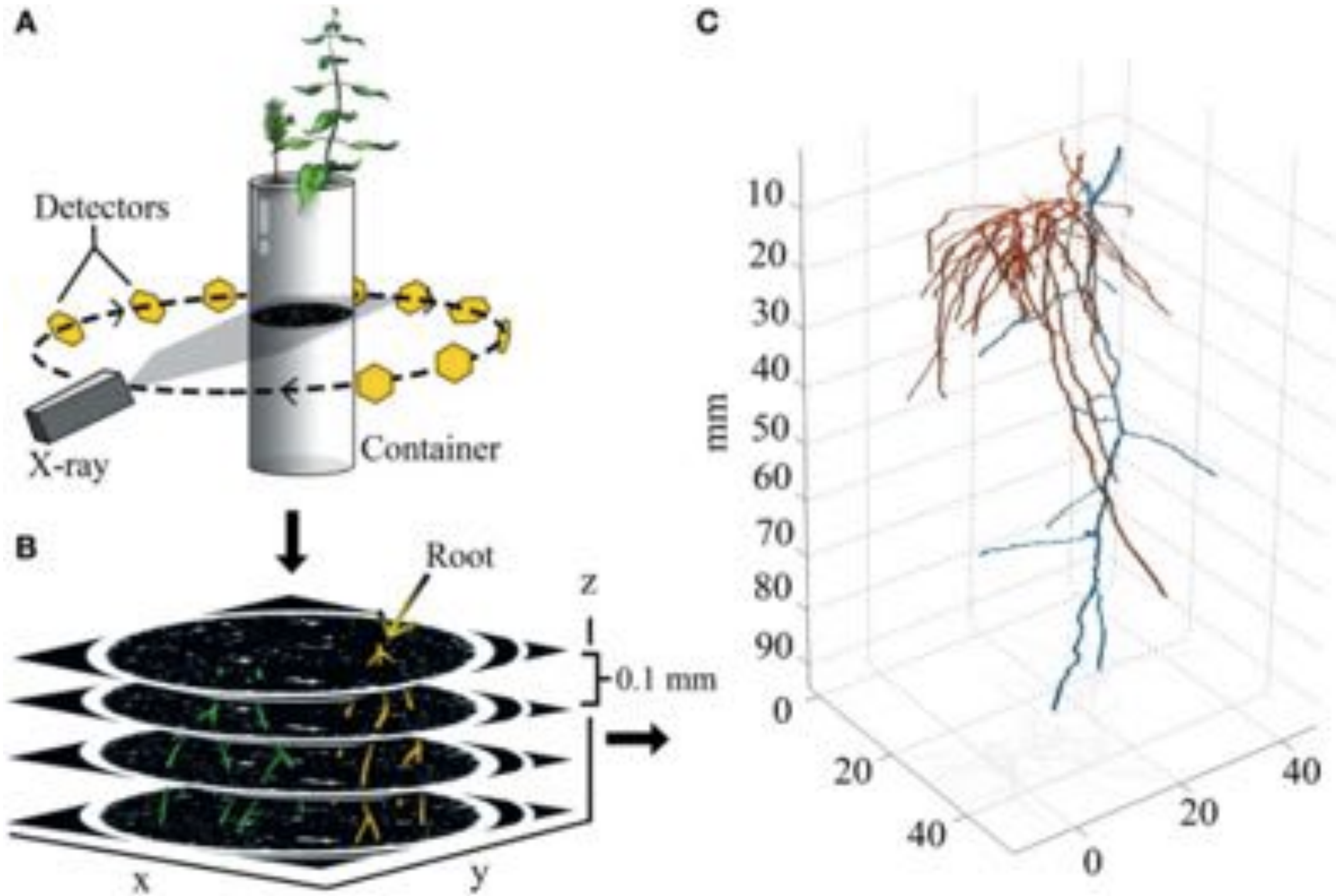
CI-600 Root Imager

X-ray computed Tomography

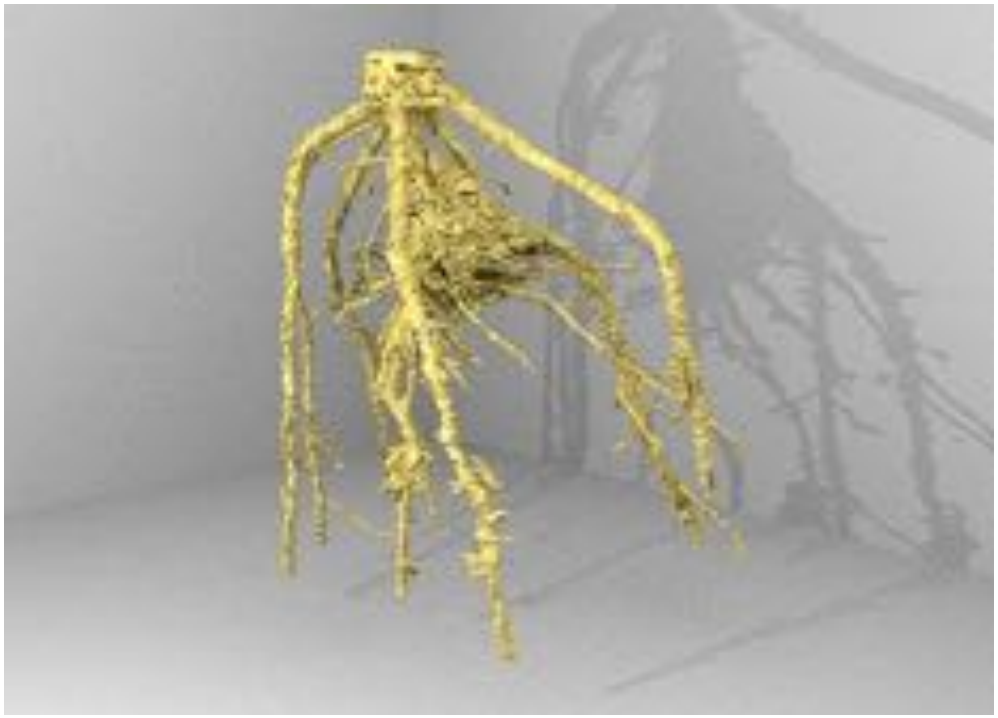
X-ray computed Tomography

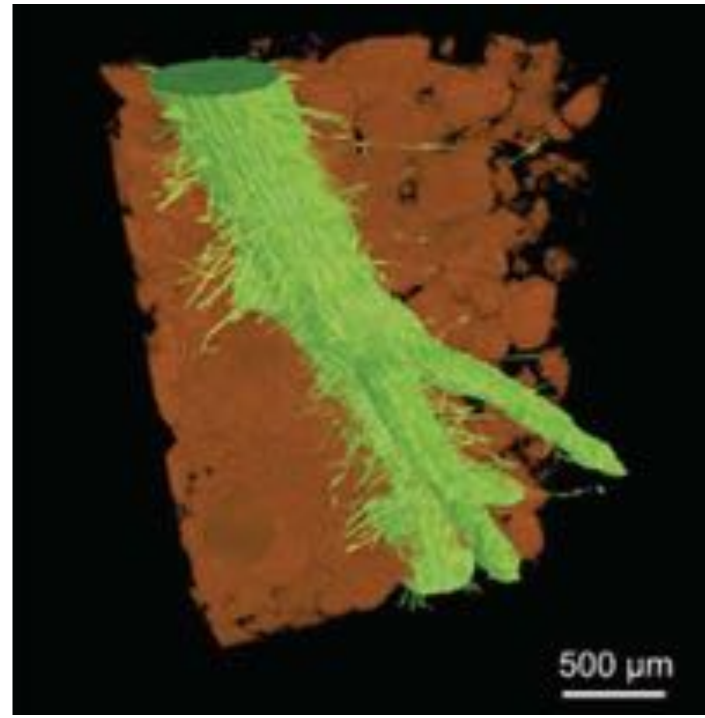
- Observation the roots and their natural state in the soil
- Several images can be obtained in a short period of time
- Several energy sources may be used
- X-ray
  - Non-invasive
  - Allow viewing inside objects in 2D or 3D
  - Principle of attenuation of electromagnetic waves

X-ray computed Tomography



X-ray computed Tomography





X-ray computed Tomography

Pros

Many measures

3D resolution

RSA evaluation

Nondestructive

Time-scaled analysis

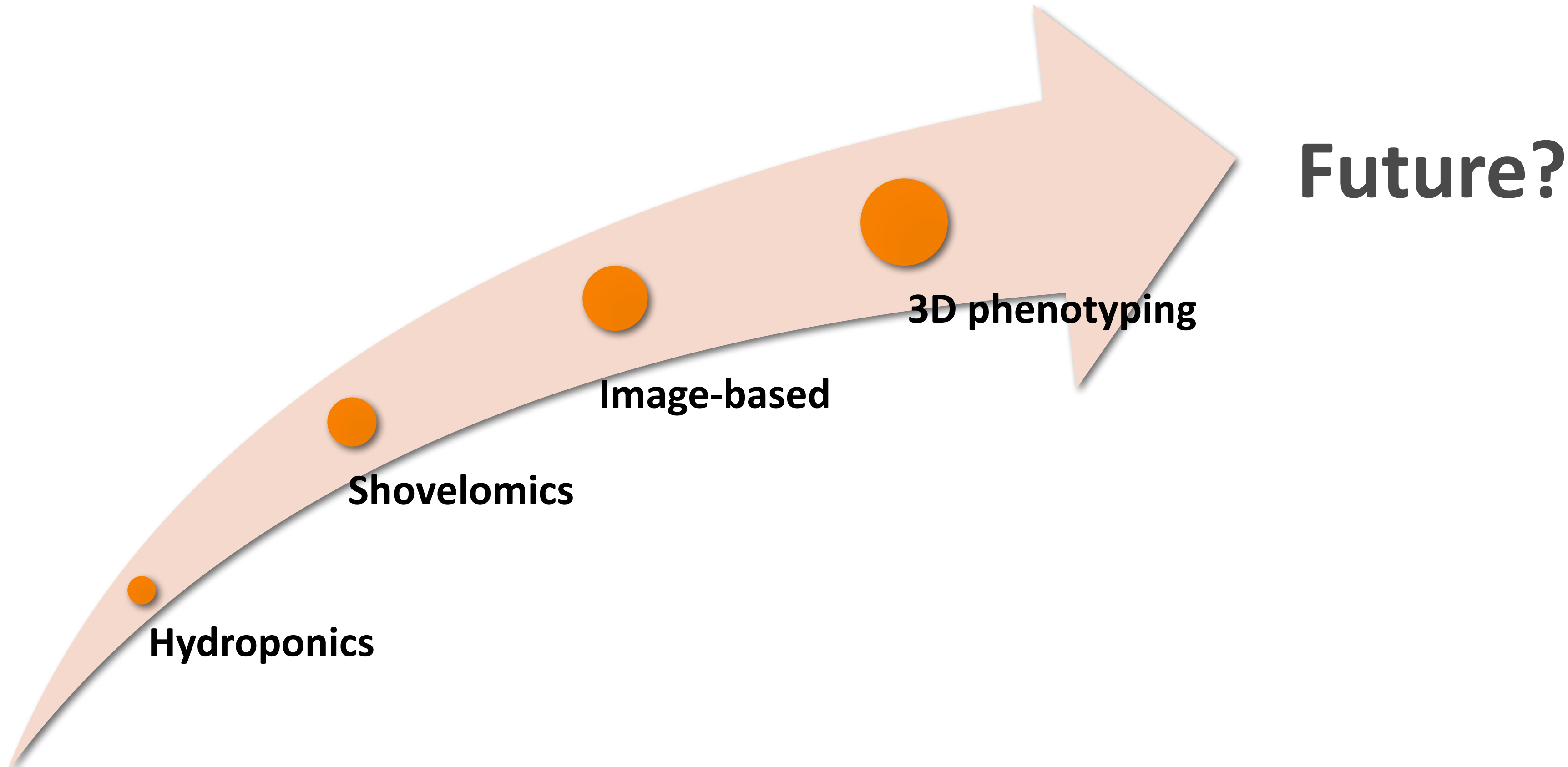
Cons

High mutation induction rate

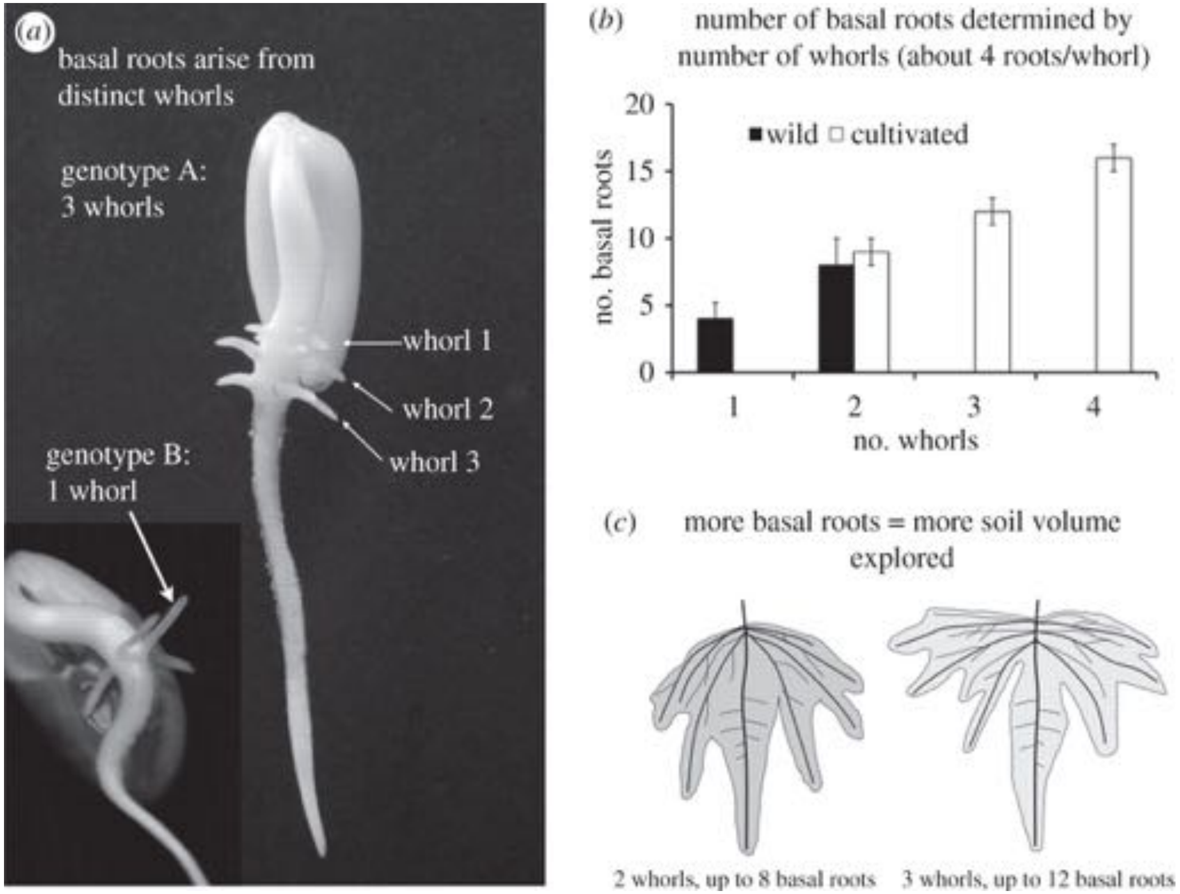
Expensive

Depends on the culture RSA

Timeline


Future?
3D phenotyping
Image-based
Shovelomics
Hydroponics

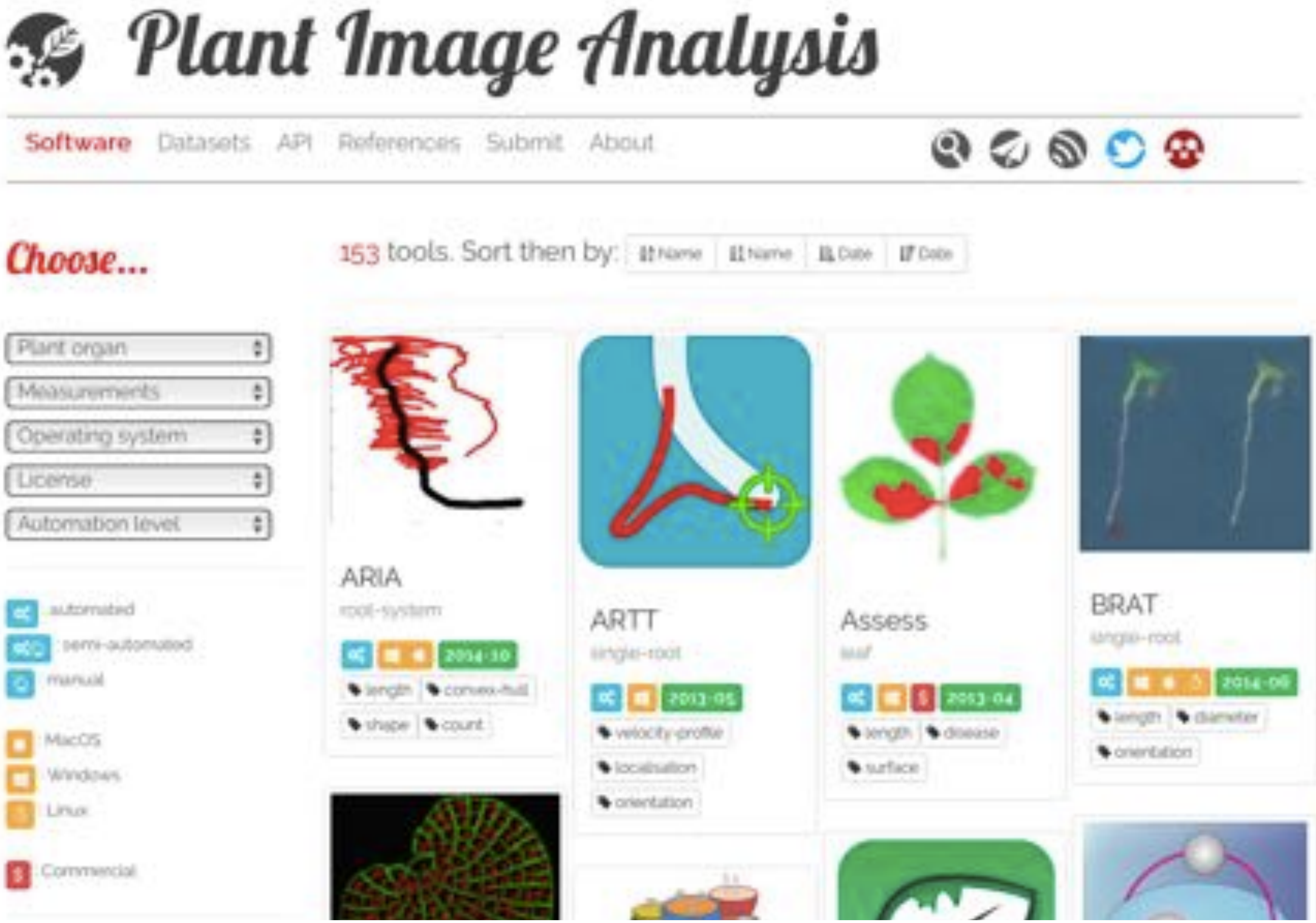
Correlation data



The future is near!

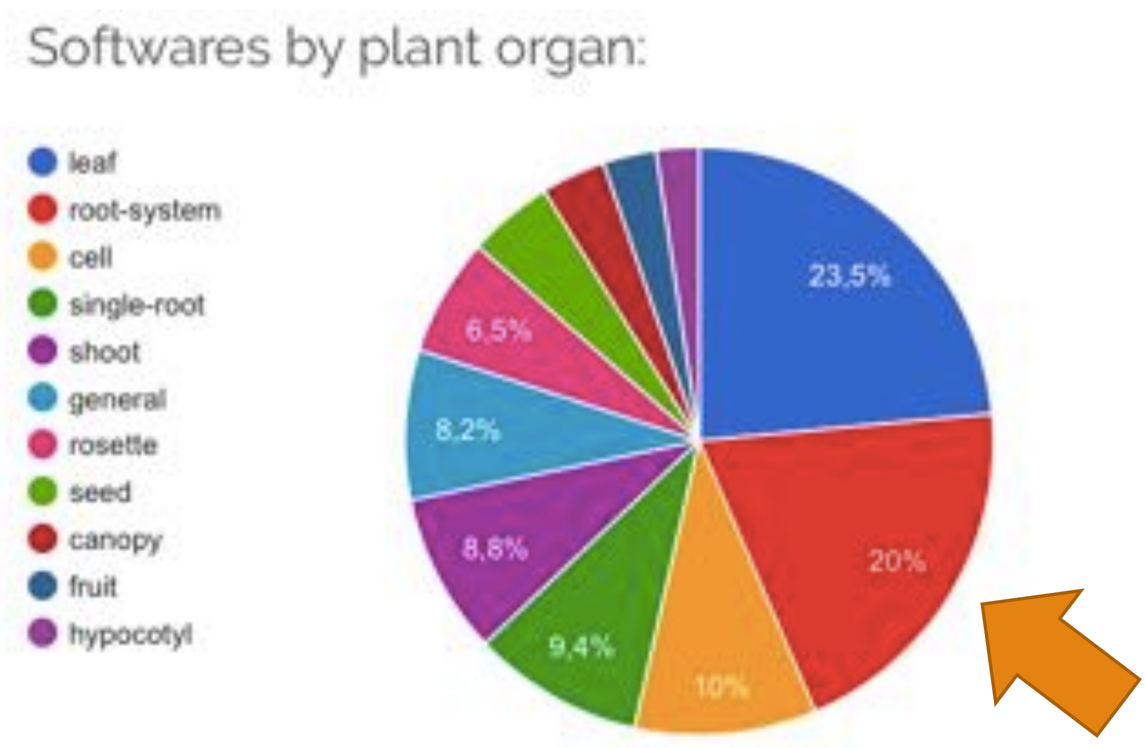
Online platform

http://www.plant-image-analysis.org



Online platform

Online platform



Investment

- Researchers in Penn State's College of Agricultural Sciences have received a **USD $7 million grant** from the U.S. Department of Energy's Advanced Research Projects Agency-Energy to design a low-cost, integrated system that can identify and screen for high-yielding, deeper-rooted crops.

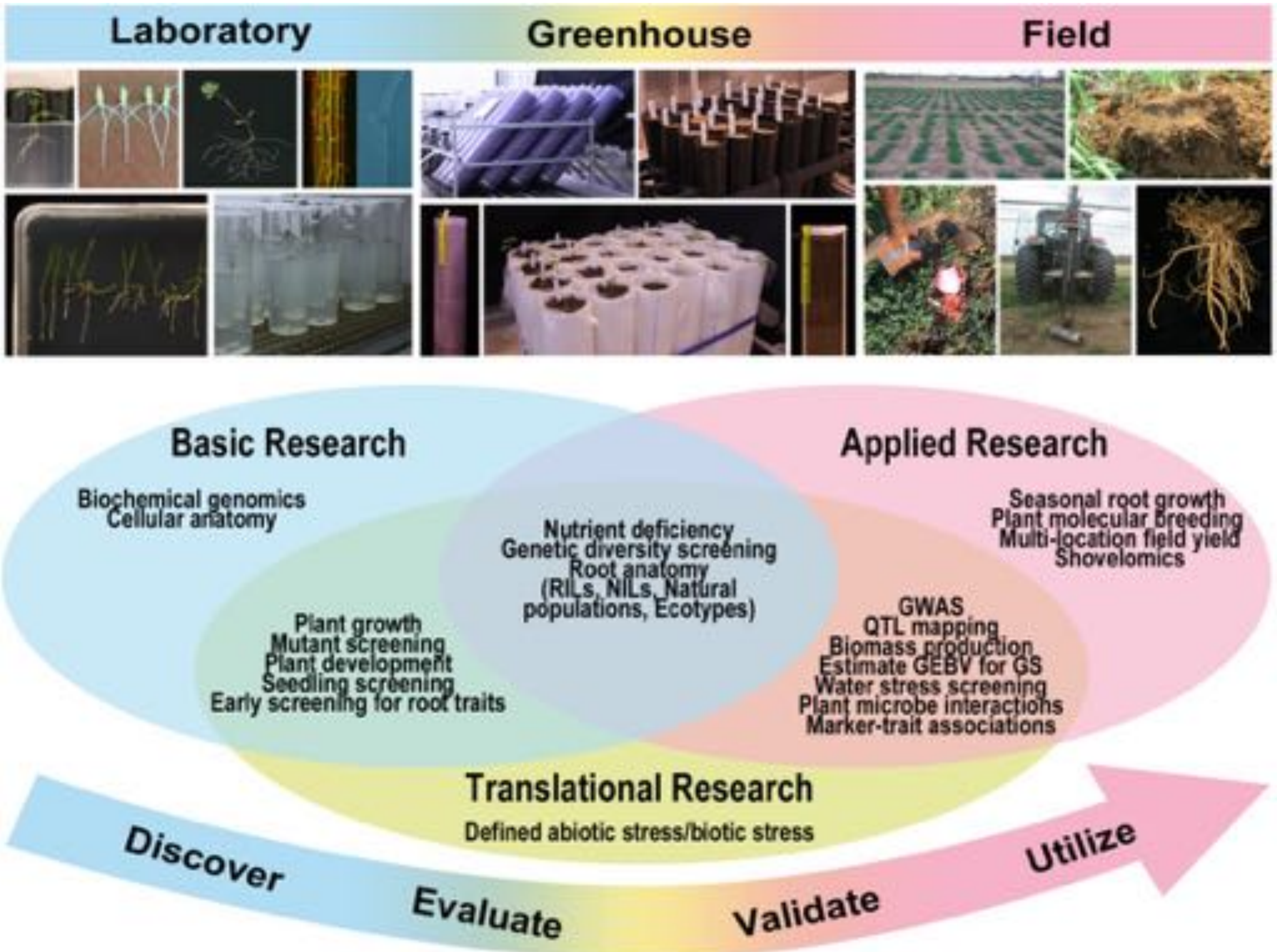
- Goal: enhancing the breeding of crop varieties better adapted for nitrogen and water acquisition and carbon sequestration.

- DEEPER: revolutionary phenotyping platform for deeper-rooted crops, which will integrate breakthroughs in:
  - non-destructive field phenotyping of rooting depth,
  - root modeling,
  - robotics,
  - high-throughput 3D imaging of root architecture and anatomy,
  - gene discovery, and
  - genomic selection modeling

Final Remarks

- There is no perfect system!
  - In accordance with objectives and conditions

Final Remarks



Final Remarks

- There is no perfect system!
  - In accordance with objectives and conditions
- Evolution to HTP
- Artificial Intelligence in root phenotyping

Thank you!