



Student Training Course

Classical and Modern Approaches in Crop Breeding
22–26 September 2025, IFVCNS, Novi Sad, Serbia

HTPP in Lab

Aleksandra Radanović

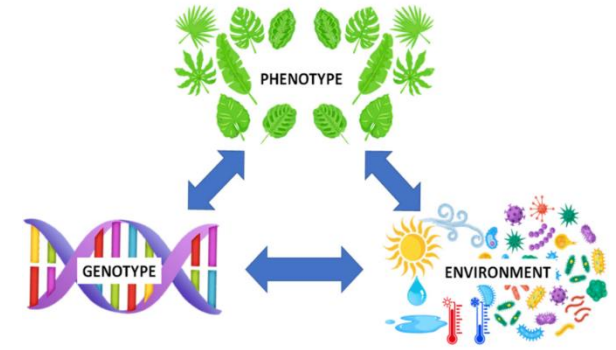




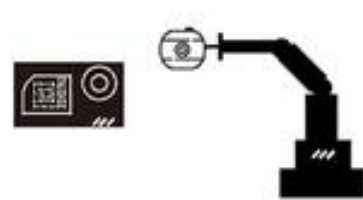
Phenotyping

Why Phenotyping Matters?

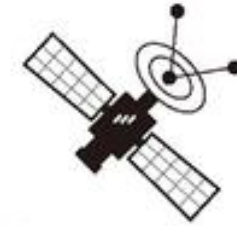
Phenotyping is the process of identifying and analyzing an organism's observable characteristics, known as phenotypes, which result from the interaction between its genetics (genotype) and the environment.



Cell scale



Plant/organ scale



Field scale



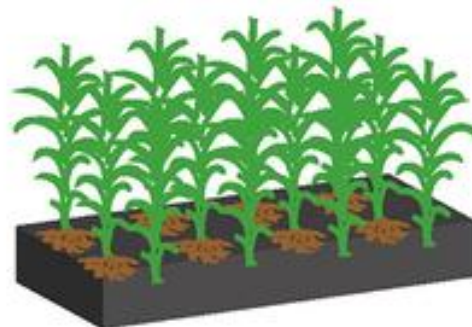
Plant cell



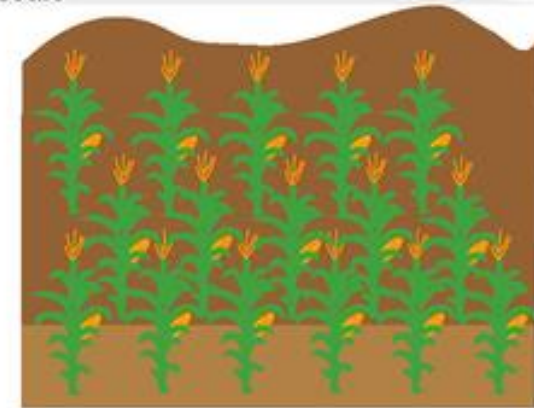
Seed

Leaf

Plant



Plot



Field



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Concept of High Throughput Plant Phenotyping (HTPP)

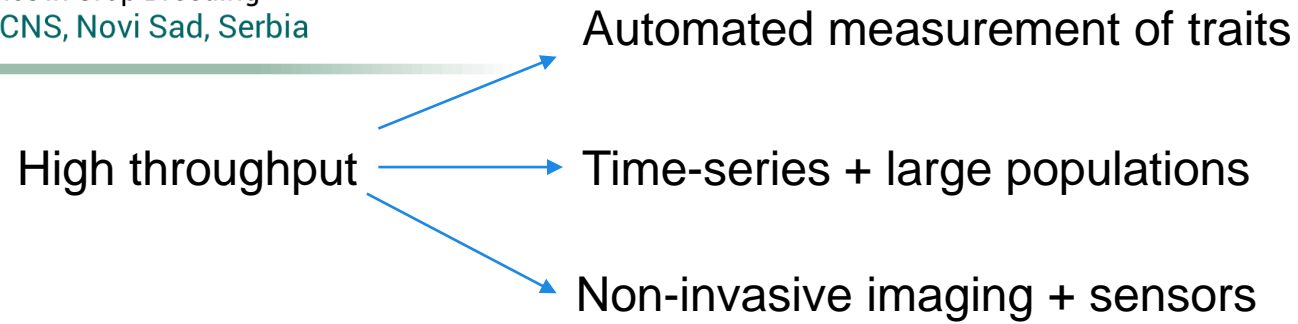
High-throughput phenotyping (HTP) is an automated and rapid method for collecting comprehensive phenotypic data from large populations of organisms, especially plants





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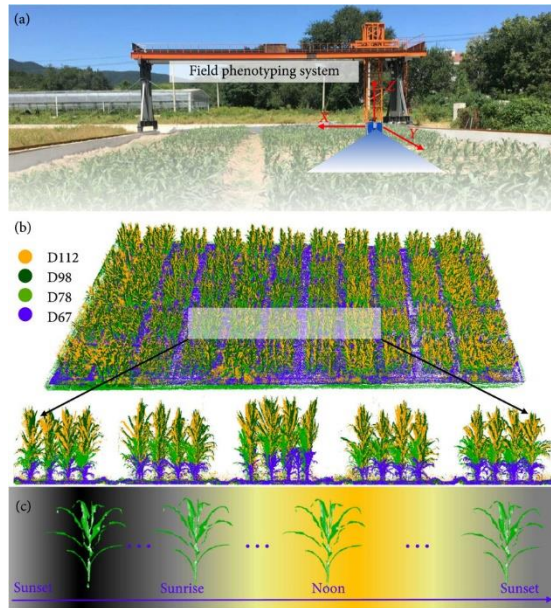


- Genetics & genomics
- Physiology & stress biology
- Breeding & screening

Two main domains:

•**Field phenotyping** (drones, tractor sensors)

•**Controlled environments** (lab, growth chamber, greenhouse)



Field: large scale, realistic, noisy



Labs: controlled, precise, smaller scale



What are advantages and limitations of Lab HTPP?

Advantages of Lab HTPP



Limitations of Lab HTPP

Controlled,
reproducible
environments

Year-round experiments

High setup costs

High precision and resolution

Less “real world” than field

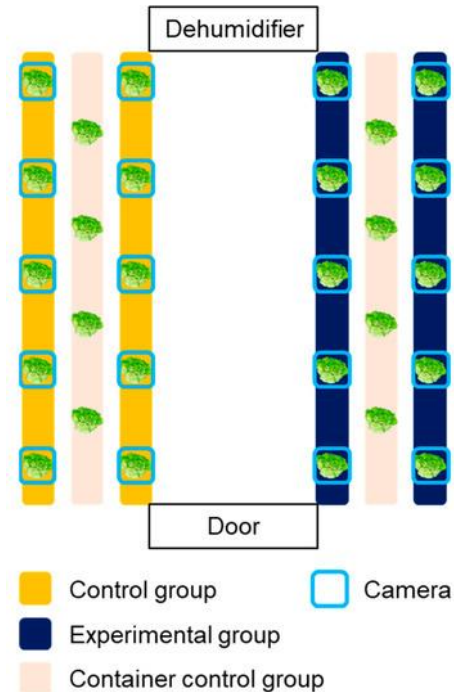
Early screening before field trials

Risk of over-controlling environment

Typical Experimental Setup

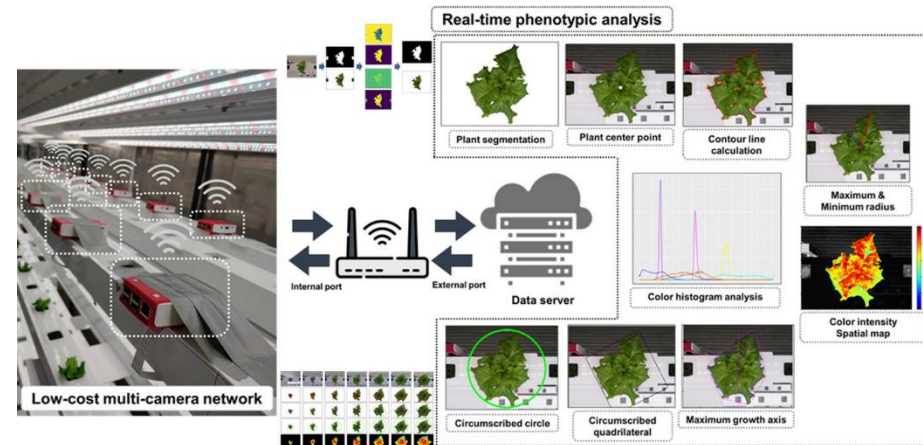
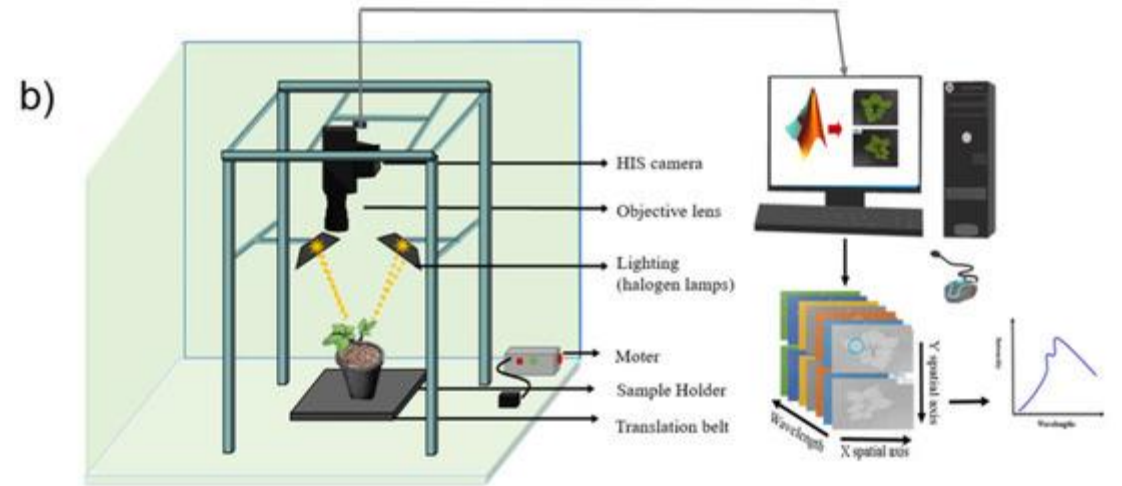
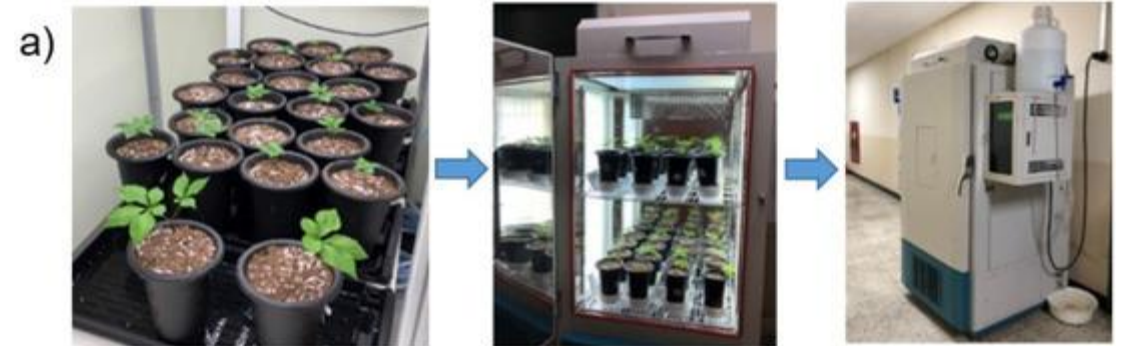
Growth chamber + automated system

- Conveyor belts moving pots
- Fixed cameras/sensors
- Automated watering and nutrient supply



<https://www.mdpi.com/2077-0472/13/10/1874>

<https://www.mdpi.com/1424-8220/21/16/5634>



Precision vs. Throughput

- **Balance between detail & scale**

High precision → low throughput

High throughput → less detail

- **Compensating for errors**

In some cases, increasing throughput can compensate for higher error rates. This is because the large sample size generated by high throughput can statistically overcome individual inaccuracies, leading to more reliable overall decisions.

- **Different platforms optimize differently**

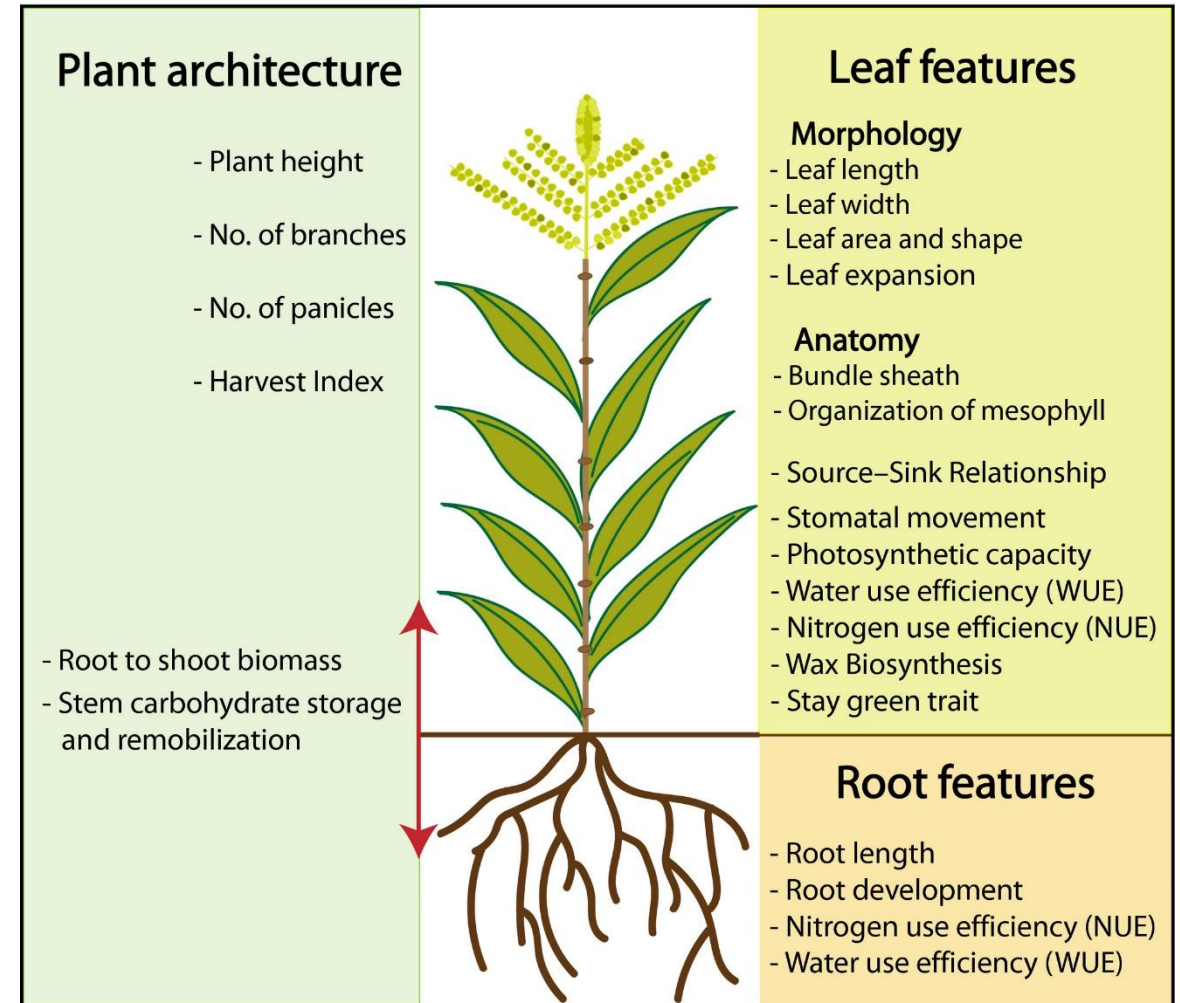
Plant breeding: Often benefits more from increased throughput, allowing for the phenotyping of larger populations and increased selection intensity.

Precision agriculture: May require higher precision to detect early signs of plant stress or disease in large fields.



Traits that can be measured

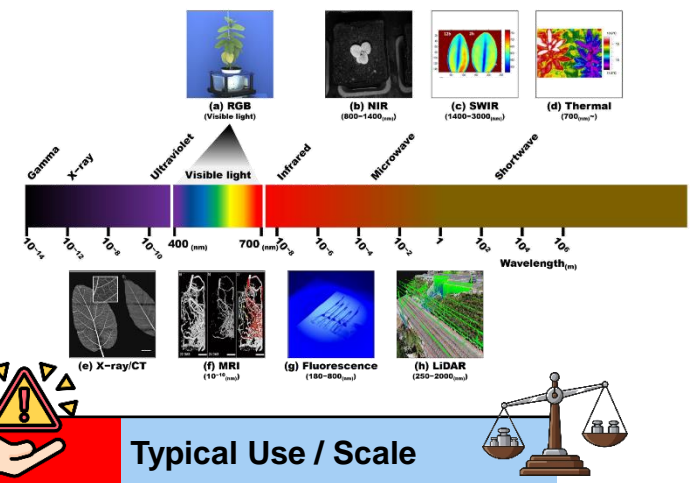
- Morphology (size, shape, and plant architecture)
- Physiology (photosynthesis, stomatal conductance, water use efficiency)
- Developmental timing (flowering etc.)
- Stress responses (wilting degree, the extent of damage from drought or disease)


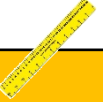







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Imaging System 	Measured Traits 	Strengths 	Limitations 	Typical Use / Scale 
RGB	Morphology (height, leaf area, architecture), color indices	Cheap, simple, high throughput, easy data processing	Limited to visible traits, sensitive to lighting, low physiological insight	Population-level screening, growth monitoring, breeding programs
Hyperspectral	Pigments, water content, stress indicators, biochemical traits	Non-destructive, precise, early stress detection	Expensive, large datasets, complex calibration	Physiological studies, stress detection, trait mapping
Fluorescence	Photosynthetic efficiency, chlorophyll activity	Sensitive to photosynthetic stress, non-destructive	Limited to chlorophyll/fluorescence traits, specialized equipment	Stress physiology, photosynthesis research, organ-level studies
Thermal / IR	Canopy temperature, transpiration, water status	Non-invasive, good for drought/water stress, moderate throughput	Sensitive to environmental variation, limited trait scope	Water-use efficiency studies, drought stress screening
3D / LiDAR	Plant architecture, biomass estimation	Detailed structural info, non-destructive	Complex analysis, equipment cost, moderate throughput	Morphology studies, biomass prediction, organ-level & whole-plant
Root imaging (Rhizotron, CT, MRI)	Root architecture, growth dynamics	Access to hidden traits, high precision	Very low throughput, expensive, specialized	Root phenotyping, functional genomics, physiology research



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Each level offers different insights:
detailed mechanisms at the organ
scale,
integrated responses at the whole-
plant scale,
and variation at the population scale..

Levels of Measurement in HTPP

- Organ (leaf)
- Whole plant
- Populations



Organ (leaf)



Whole plant



Populations





Phenotyping Across Time

Static Measurement

- Single time point
- Snapshot
- Specific trait



Dynamic Measurement & Time-Lapse

- Continuous monitoring
- Growth curves
- Developmental trajectories
- Early response detection

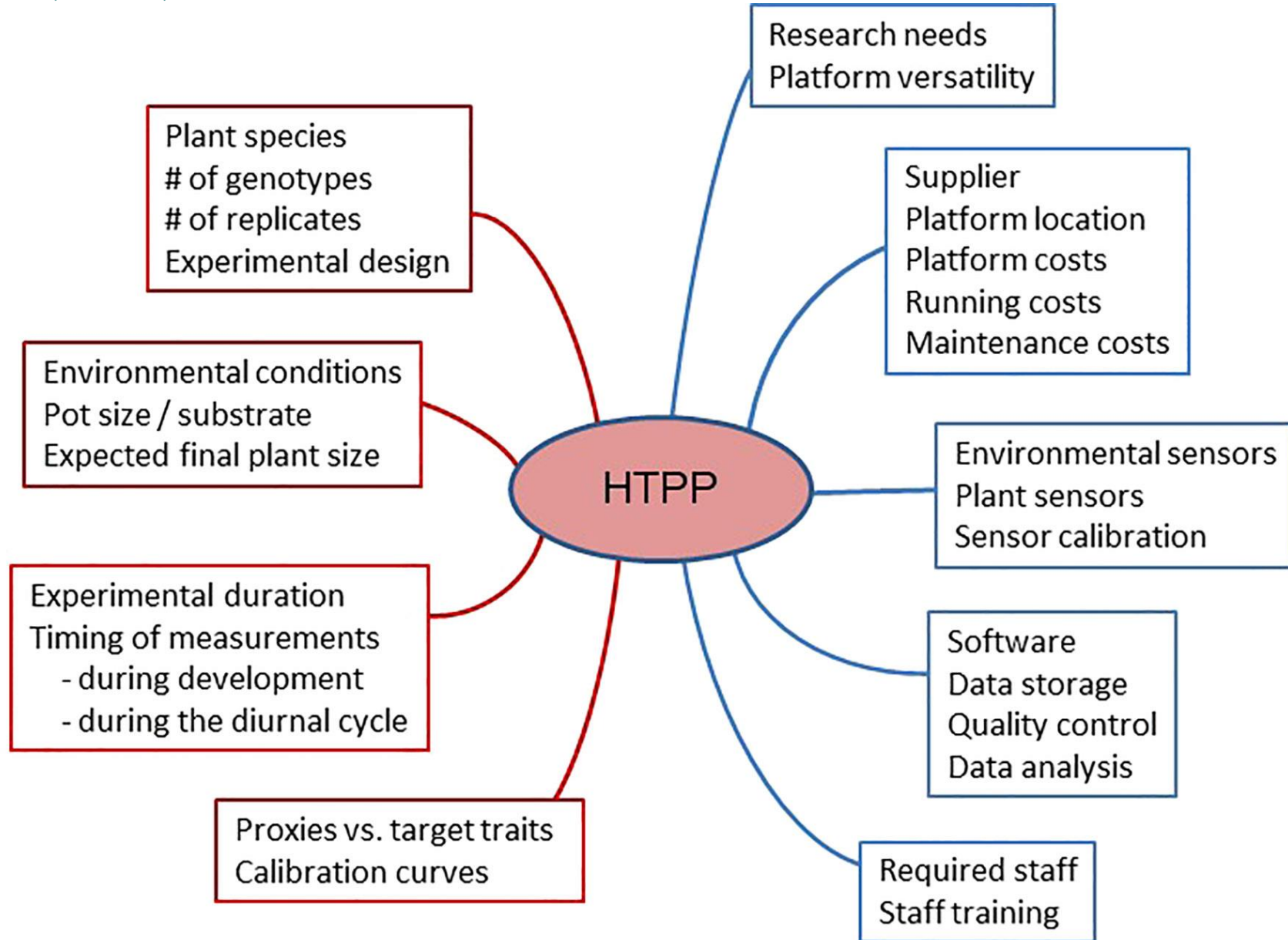




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Different aspects
HTPP systems that
should be considered
before purchasing
such a system (in
blue) and using it (in
brown).



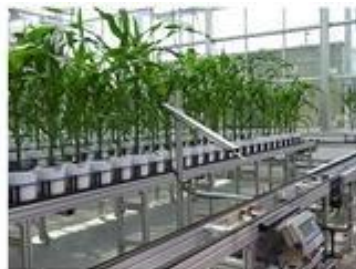


Commercial Systems

LemnaTec Scanalyzer



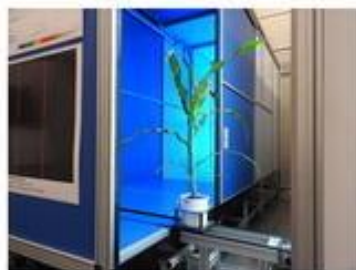
a



b



c



d

<https://www.youtube.com/watch?v=VVi5CbFV928>

Phenospex PlantEye



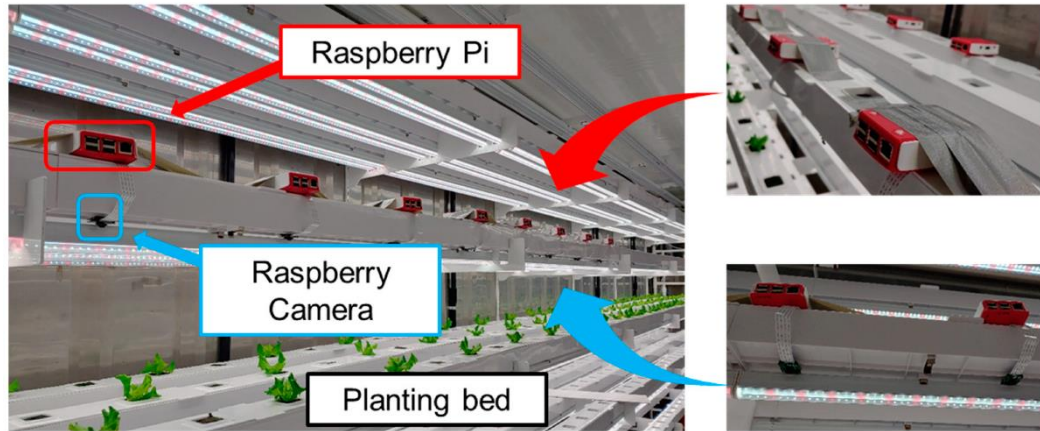
<https://www.youtube.com/watch?v=XoFc9TcSXJI>



Photon Systems Instruments (PSI)

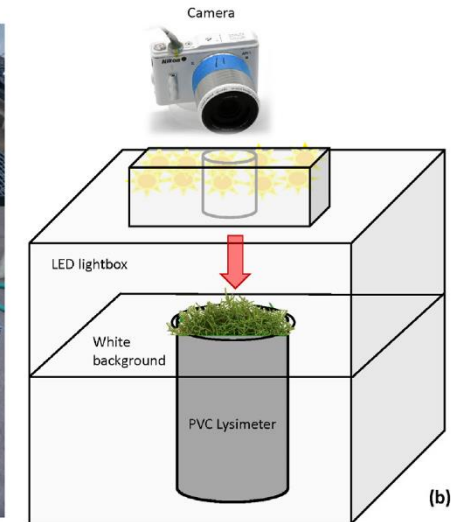
Low-cost Solutions

Raspberry Pi + cameras



- **Hardware-driven** low-cost imaging.
- Automated data collection
- Self-contained: Pi = computer + camera + optional lights/sensors.
- Requires some coding (Python, OpenCV).
- Focus: **automating image capture** in simple setups.

DIY imaging boxes



- **Environment-driven** setups (light box, chamber, or cabinet).
- Camera can be Pi, webcam, DSLR.
- Focus: **image quality, reproducibility, controlled lighting/background.**
- More flexible hardware, but usually needs external PC/software to process.



Open-source software pipelines

ImageJ / Fiji

- Language: Java
- Purpose: General image analysis (not plant-specific, but heavily used).
- Features:
 - Plugins for area, perimeter, color histograms.
 - Segmentation of leaves, roots, seeds.
 - Works for single images or small datasets.
- Website: <https://imagej.net/software/fiji>

Phenopipe

- Language: R
- Purpose: Automates phenotypic trait extraction using ImageJ macros inside R.
- Features:
 - Workflow reproducibility.
 - Links image analysis directly with statistical analysis.
- Website:
<https://github.com/phenopipe/phenopipe>



Open-source software pipelines

🌿 PlantCV

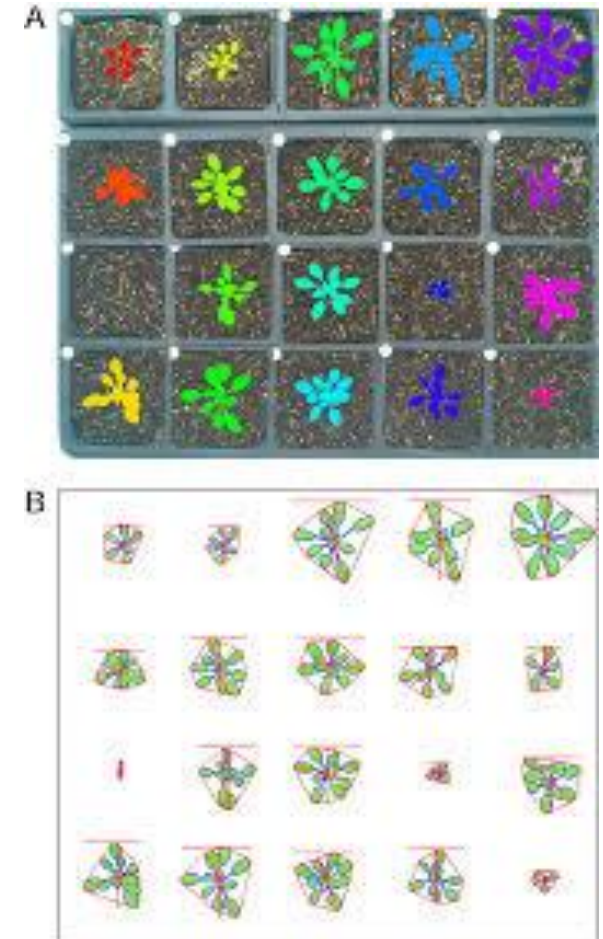
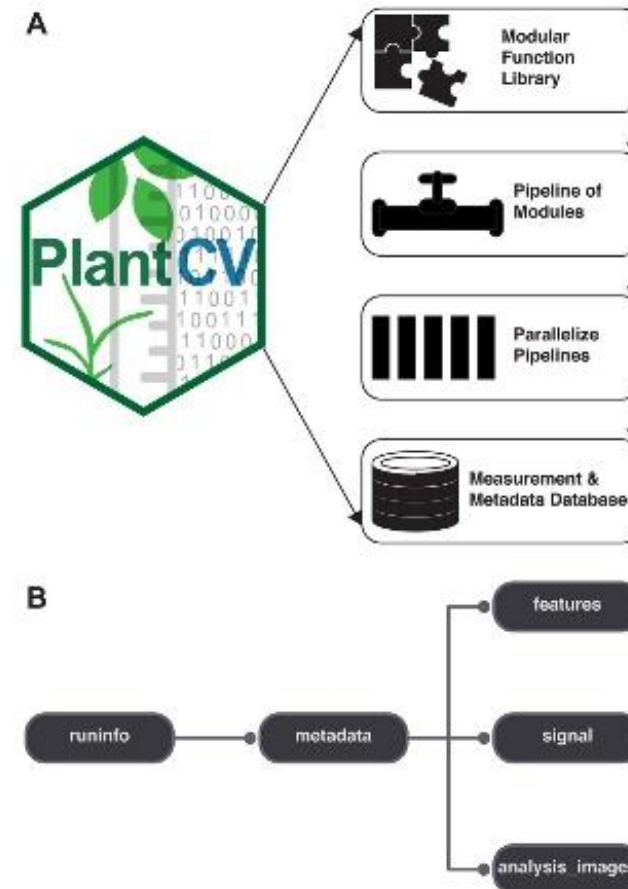
Language: Python

Purpose: Comprehensive image analysis - huge library of plugins for morphology, color, hyperspectral, fluorescence.

Features:

- Modular pipeline (you chain functions).
- Batch processing of large datasets.
- Works with Raspberry Pi/DIY box images as well as commercial datasets.

Website: <https://plantcv.danforthcenter.org>



<https://peerj.com/articles/4088/>

http://plantcv.readthedocs.io/en/latest/analysis_approach/

Open-source software pipelines

🌱 RootPainter

- Language: Python (Deep Learning) - software tool for the rapid training of deep neural networks for use in biological image analysis
- Purpose: Semi-automated root segmentation.

• Features:

- Interactive AI model training.
- High accuracy root extraction from images.

• Website:

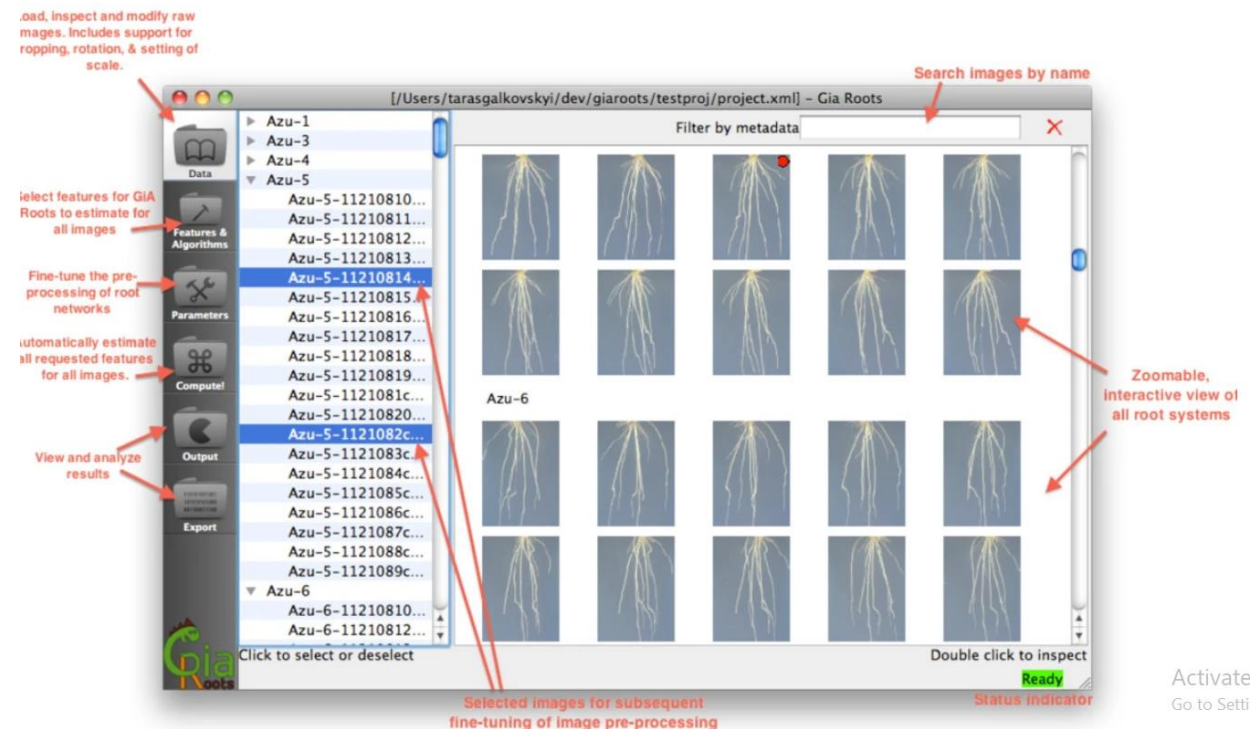
<https://github.com/Abe404/rootPainter>

<https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18387>

<https://bmcplantbiol.biomedcentral.com/articles/10.1186/1471-2229-12-116>

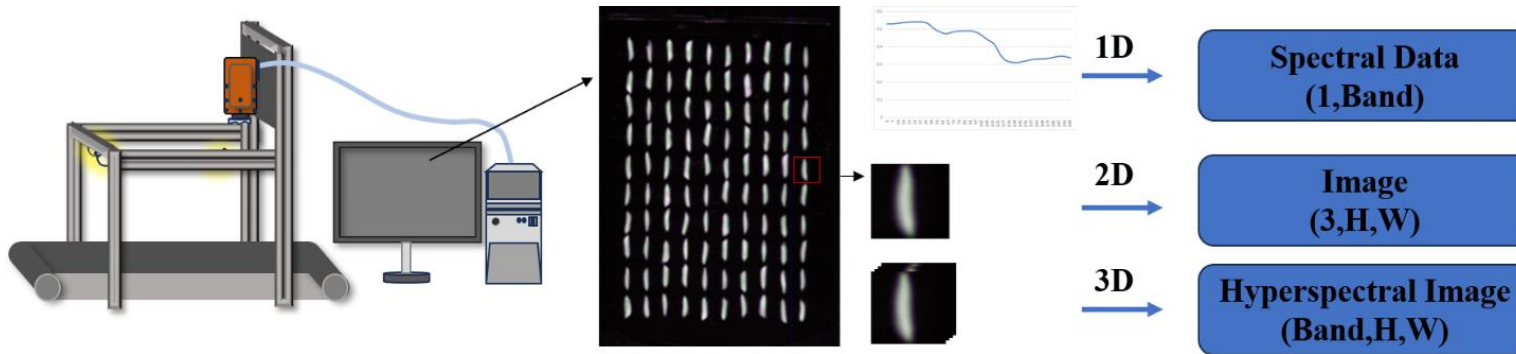
GiA Roots

- Language: ImageJ plugin
- Purpose: Root system architecture analysis.
- Features:
 - Extracts traits like length, branching, angle.
- Website: <https://github.com/GIA-Roots>



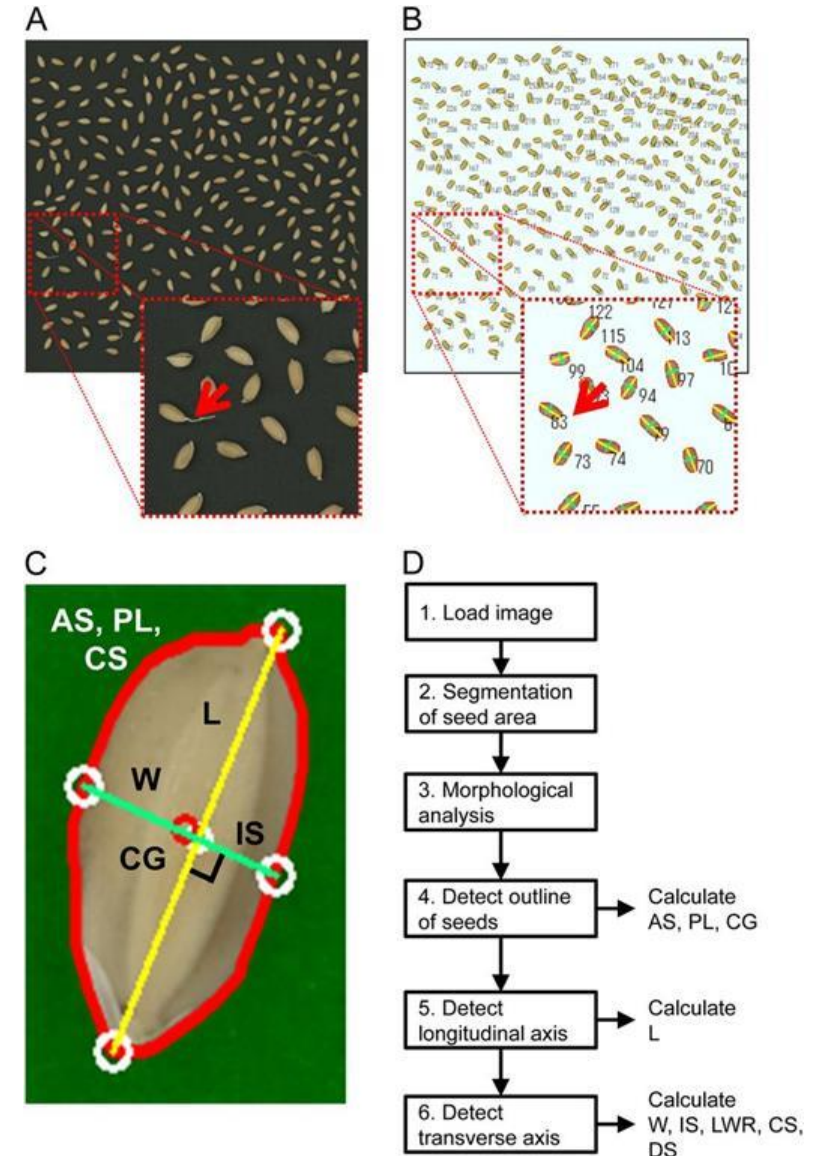
Examples of Seed HTP

Seed shape and size are among the most important agronomic traits because they affect yield and market price



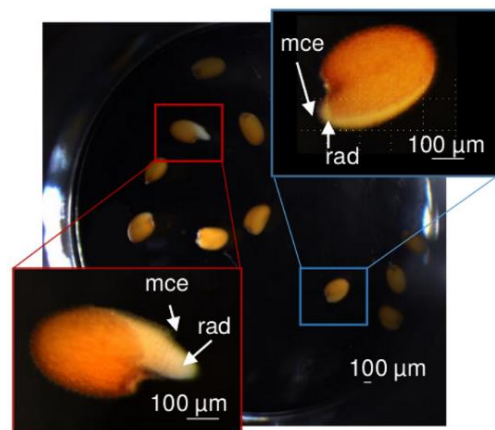
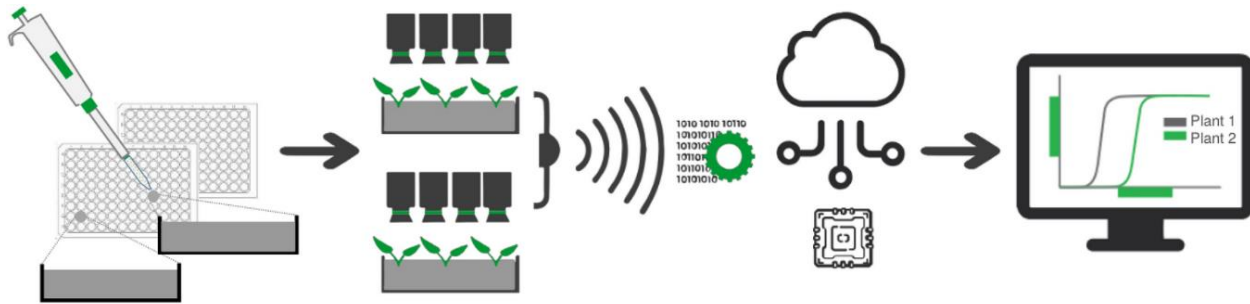
Reduced sampling error and made it possible to distinguish between lines with small differences in seed shape

SmartGrain: High-Throughput Phenotyping Software

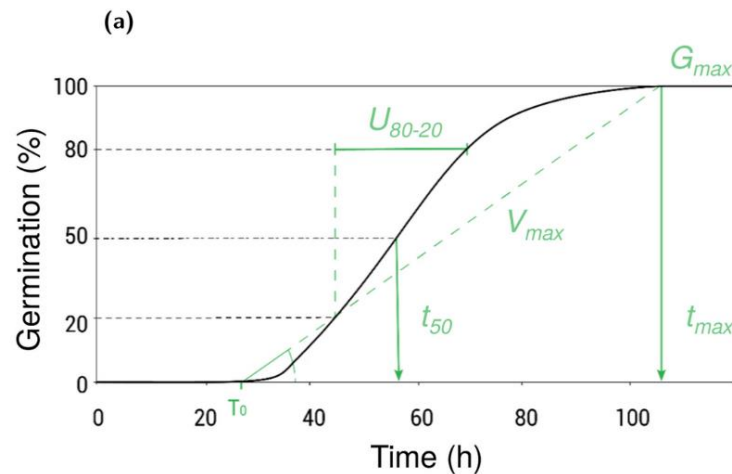


Examples of Seed HTP

ScreenSeed



(b)



(c)

Workflow.

(a) **Seeds are sown by a pipetting method** in microplates that are deposited in an automate
Pictures taken hourly
Images are transferred by Internet connection to a database for computational analysis and seed germination scoring.
 The software processing provides germination kinetics and extracts metrics with representation systems and statistical analyses that help to compare seed quality in a dashboard.

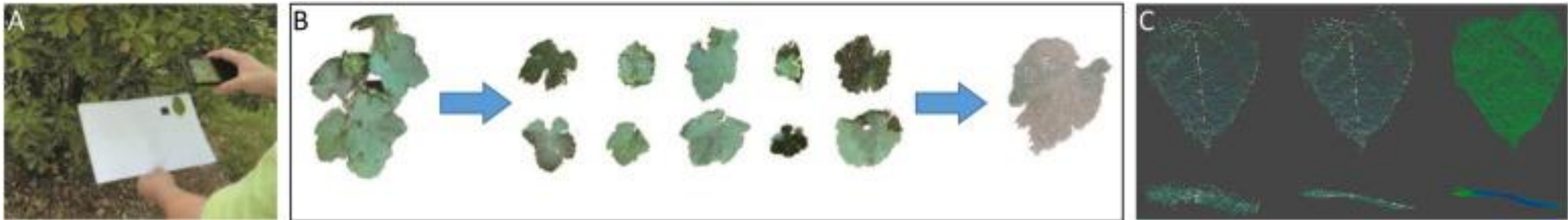
(b) **Imaging detection of germinated seeds. The imaging software is trained to score germinated seeds in microplate wells.** In the microplate wells, the software identified germinated seeds among the detected seeds.

(c) **Metrics of germination kinetics provided by the ScreenSeed dashboard.** The kinetic curve is represented by cumulative germination percentage scored every hour during seed hydration. The metrics G_{max} (maximal percentage of germination), U_{80-20} (80/20 time spread it the time interval between 20% and 80% germination), t_{50} (time required to reach 50%) are automatically extracted from the database and can be compared between samples.

Examples of Leaf HTP

Leaf area can be studied for a single leaf, a whole plant, or a group of plants.

The leaf area of a group of plants in the field is commonly quantified by leaf area index (LAI)



Application of modern imaging technology for leaf-area measurement at several scales:

- (A) Digital camera acquires the image of a single leaf and a calibration object
- (B) Grape leaf extraction, point-cloud segmentation and surface reconstruction
- (C) 3D point-cloud and surface reconstruction of a single leaf



Examples of Leaf HTP

A rapid and non-destructive leaf area measurement system for **Android phones** -take a picture of a leaf next to a reference object of known size → mobile app like **Leaf-IT** or **Petiole** to process the image

The app performs digital image processing, including margin detection and pixel counting, to calculate the leaf's area by comparing its pixel values to those of the reference object.



Ensure the entire leaf and calibration plate are within the frame.



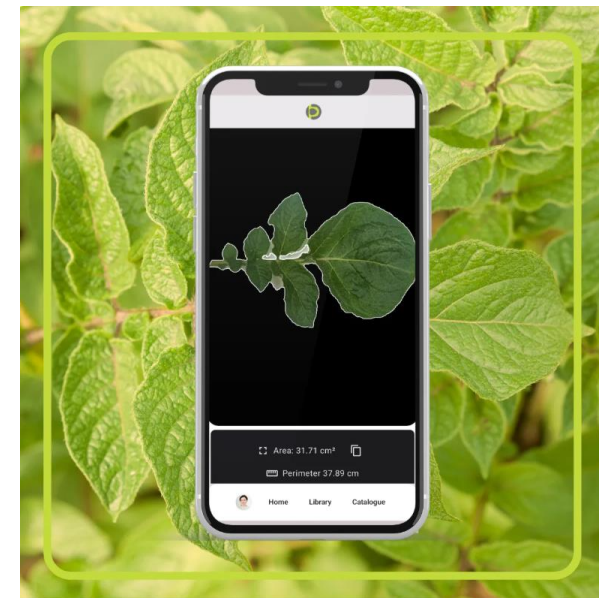
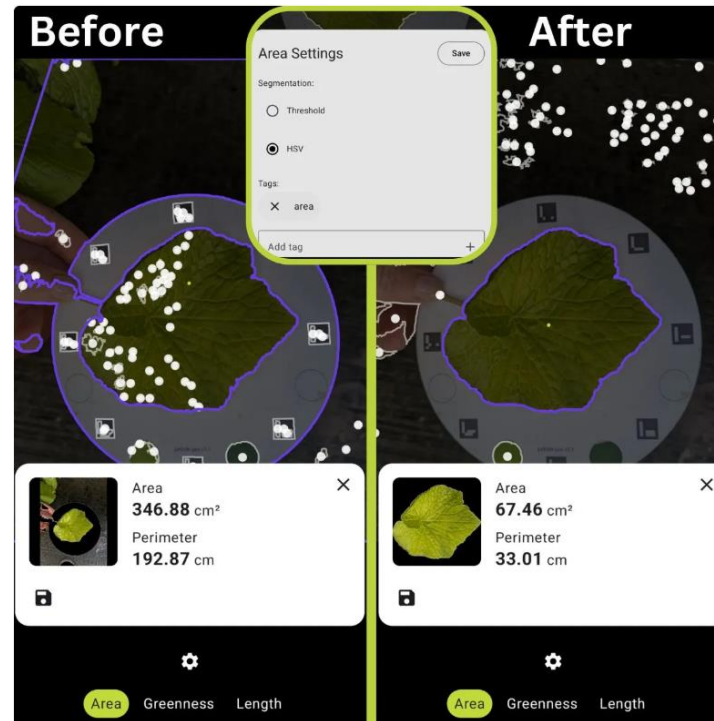
Maintain steady positioning and good light while capturing the image.

- Rapid and Non-Destructive:** Allows for quick measurement without damaging the leaf.

- Portable:** Measurements can be taken anywhere, increasing flexibility for remote locations.

- Accuracy:** Systems like Leaf-IT have shown high accuracy and precision, with error rates comparable to established commercial software.

- User-Friendly:** Android applications make the process easy to use.



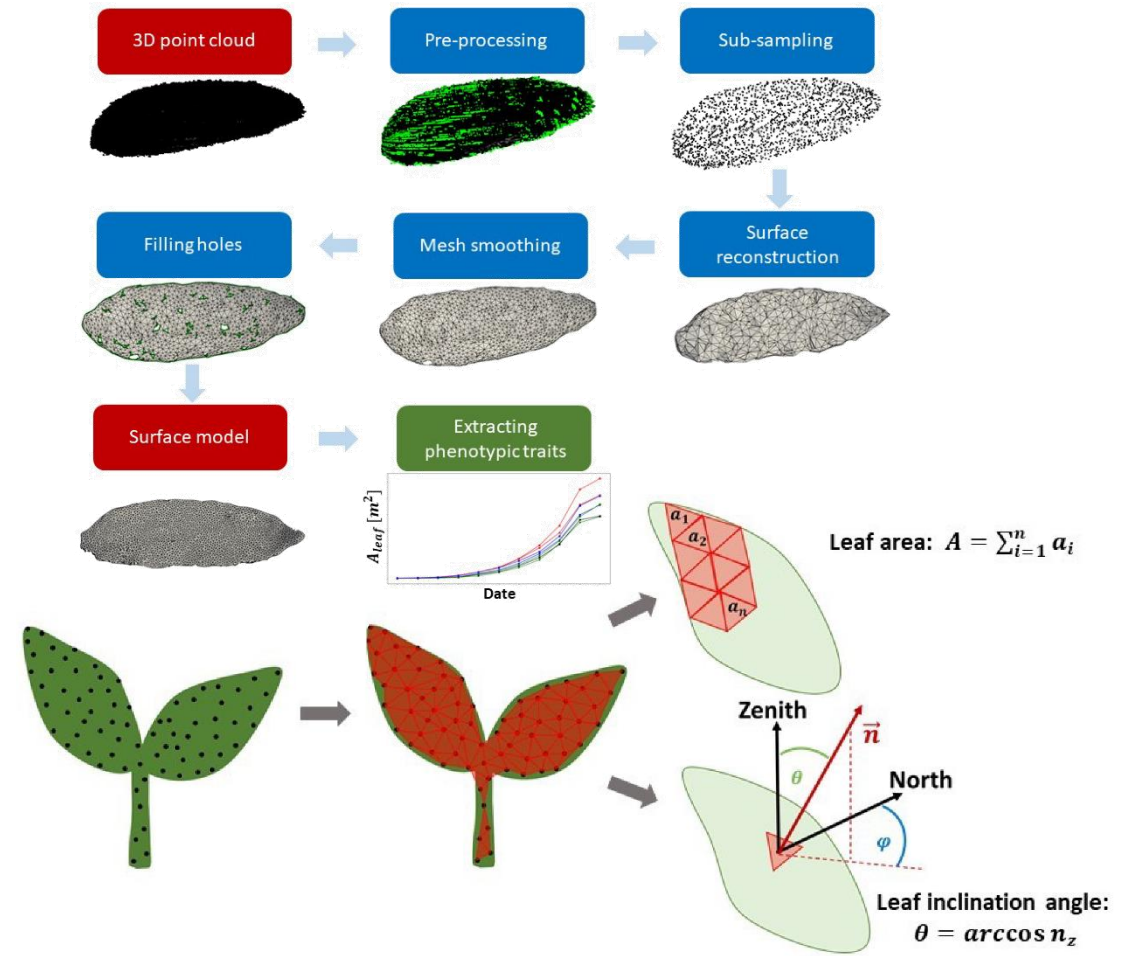
<https://www.petiolepro.com/blog/a-comprehensive-guide-for-non-destructive-leaf-area-measurement/>

Examples of Leaf HTP

- Leaves are **curved, folded, or overlapping** (e.g., maize, sorghum, sunflower, tobacco).
- For thaits: **leaf angle, curvature, plant architecture**.

• **The pipeline steps:** pre-processing, outlier removal, subsampling, then surface reconstruction, smoothing, hole filling. Especially because these steps determine accuracy.

- Start from a **3D point cloud** (generated by laser scanning, photogrammetry, or depth cameras).
- **Noise removal** → filters out stray points (caused by reflections, shadows, sensor errors).
- **Leaf segmentation** → clustering algorithms group points belonging to the same leaf.
- **Surface reconstruction** →
 - Ball Pivoting Algorithm (BPA) “wraps” the points with triangles to create a mesh.
 - Produces a smooth, continuous leaf surface.
- **Leaf area calculation** → sum of all tiny triangle areas in the mesh = total leaf area.





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Examples of Root HTP

Preparation



Monitoring



Harvest



Screening



https://www.youtube.com/watch?v=hQ41B0zoRWY&list=P Lr-224SRPzmQM5wy8n_9Qi1kSayl__b7u

Examples of Root HTP

Almost 900 rhizotrons with a size of 80x40x5 cm.

Automatisation of all steps

Roots have **complex branching patterns**, fine root hairs, and irregular backgrounds that challenge classical image processing.

Introducing AI in image analysis

•Advantages

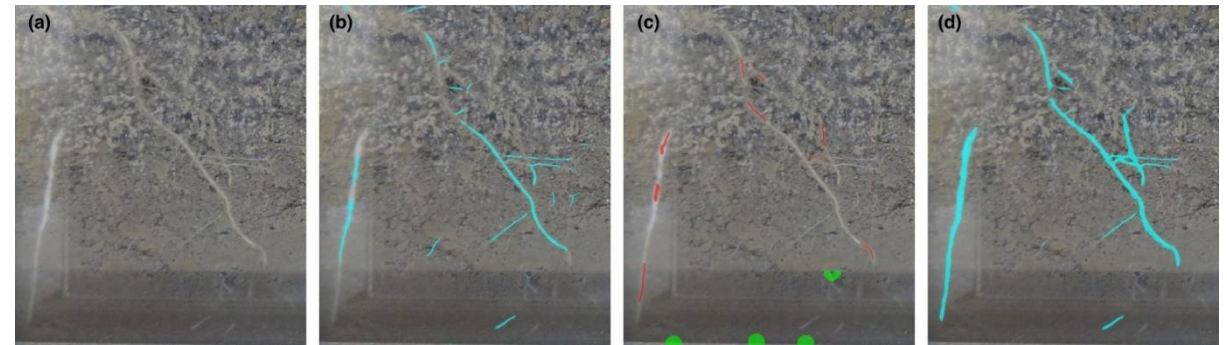
- Faster and more reproducible than manual annotation.
- Captures subtle traits (e.g., fine root density, branching angles).
- Reduces human bias.

•Challenges

- Requires annotated training data.
- Models may not generalize well across soil types, imaging setups, or species without retraining.
- Needs computational resources (GPUs for training).



<https://www.fz-juelich.de/en/ibg/ibg-2/expertise/technology-process-development/growscreen-rhizo-3>

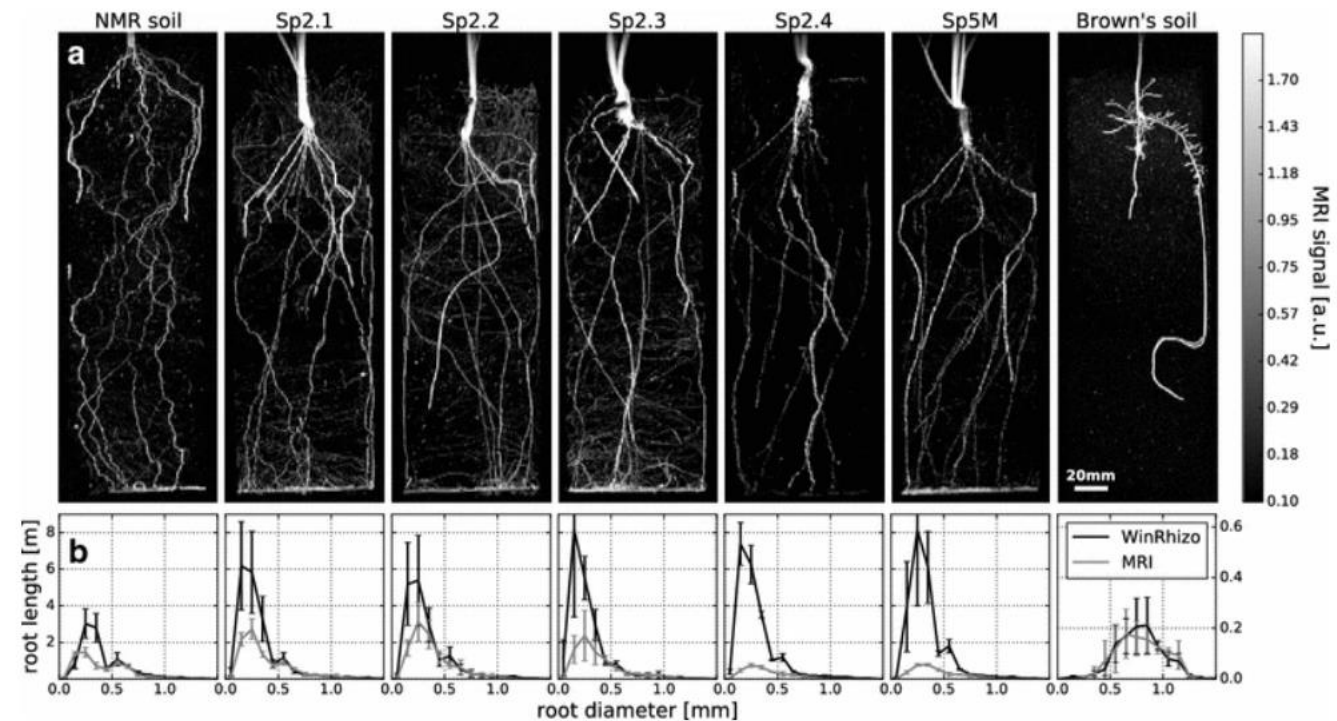


<https://nph.onlinelibrary.wiley.com/doi/10.1111/nph.18387>

Examples of Root HTP

Magnetic Resonance Imaging (MRI) in Root Phenotyping

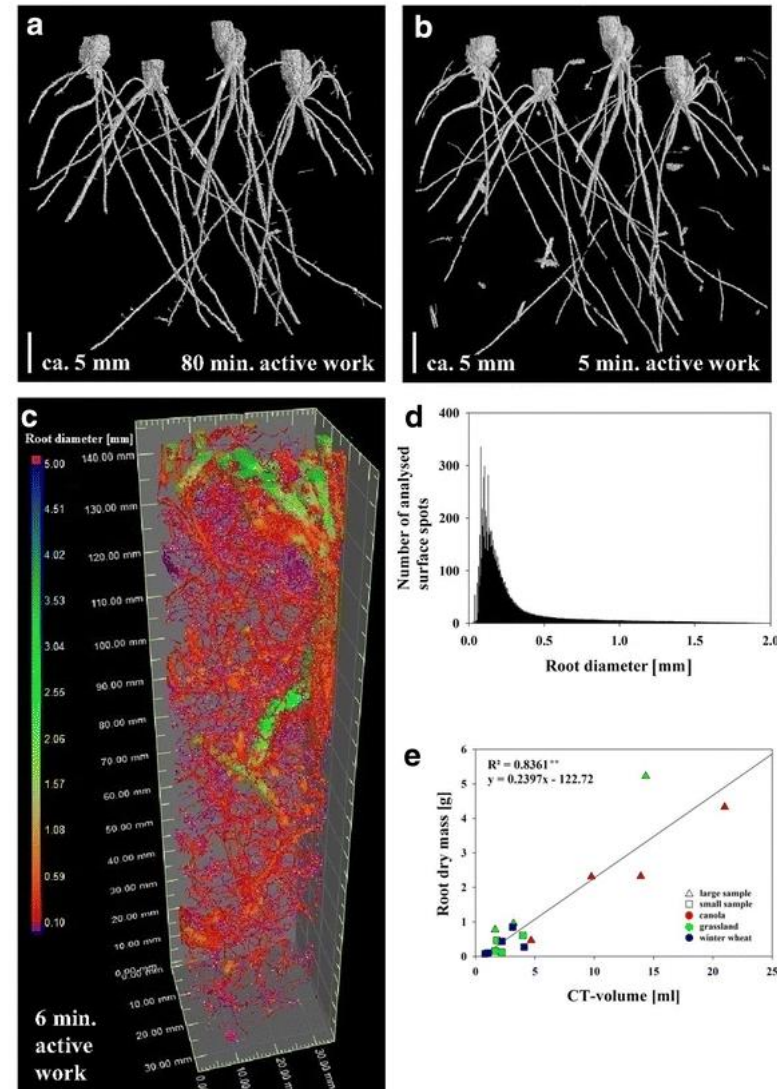
- **Non-destructive imaging** of root systems in soil or gel.
- Uses magnetic fields and radio waves to visualize water content → roots are clearly contrasted against soil.
- Enables tracking of **root growth dynamics over time** in the same plant.
- Excellent for **fine roots and root hairs**, especially in controlled environments.
- **Limitations:**
 - Expensive equipment.
 - Limited resolution in dense soils.
 - Mostly restricted to lab-scale studies.



Examples of Root HTP

X-ray Computed Tomography (CT) in Root Phenotyping

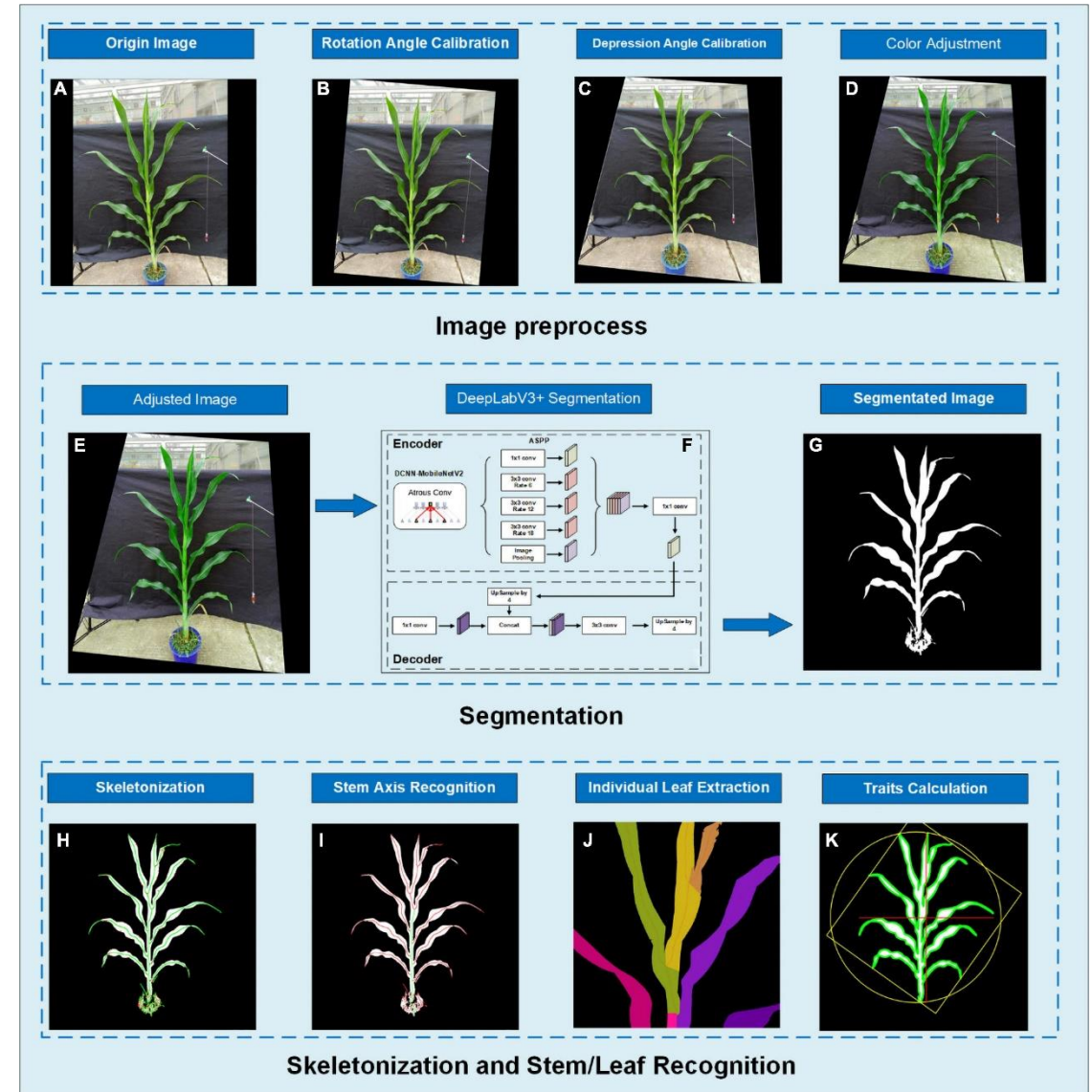
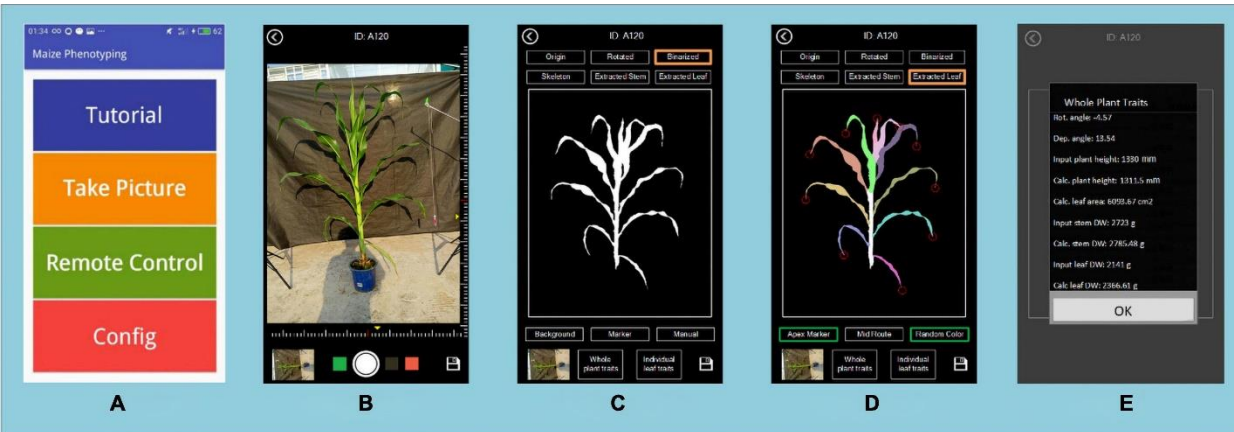
- Produces detailed **3D reconstructions** of roots inside soil columns or pots.
- Works by passing X-rays through the sample and reconstructing density differences.
- Captures complex traits: **root length, diameter, branching, root–soil interactions.**
- Useful for crops with **thick or highly branched roots** (maize, wheat, barley).
- **Limitations:**
 - High cost and limited sample size (usually pots, not field).
 - Resolution decreases with larger soil volumes.
 - Radiation safety requirements.



Examples of Whole-Plant HTP

PocketMaize: An Android-Smartphone Application for Maize Plant Phenotyping

Measures up to 45 traits:
15 plant traits,
25 leaf traits and 5 stem traits, based on images.





Examples of Whole-Plant HTP

A

Depression Angle: -2.1° Rotation Angle: 4.9°		Depression Angle: 8.4° Rotation Angle: 5.7°	
Depression Angle: 15.2° Rotation Angle: 4.5°		Depression Angle: 22.7° Rotation Angle: 3.8°	

Comparison of the images taken with different depression angles and their angle calibrated results showing a potted sample

Evaluation of an intelligent artificial climate chamber for high-throughput crop phenotyping in wheat

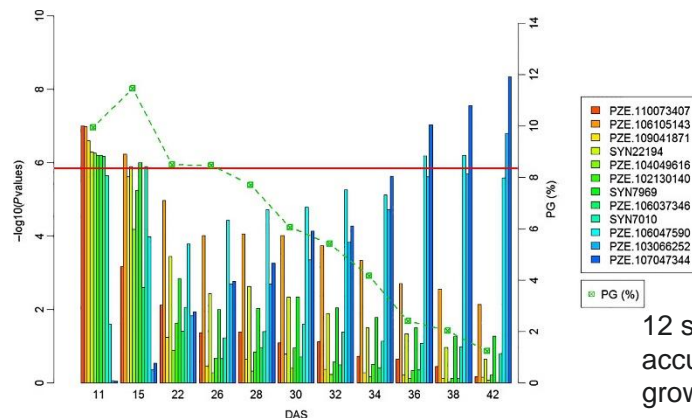


Schematic of the overall structure of the intelligent artificial climate chamber

Examples of Population HTP

Genetic variation of growth dynamics in maize (*Zea mays* L.) revealed through automated non-invasive phenotyping

- Objective:** Investigate the genetic basis of growth dynamics in maize using high-throughput, non-invasive phenotyping.
- Approach:** Monitored growth trajectories of 252 diverse maize inbred lines - 11 different time points during vegetative growth in three successive cultivations
- Technology:** Employed automated imaging systems to capture growth data, enabling precise tracking of plant size and development.
- Corellation with field data significant.**



12 significant marker-trait associations with accumulated biomass at different individual growth time points.



Key Applications of AI/ML in Crop Phenotyping

1. Trait extraction from images:

1. Leaf area, plant height, biomass, stress indicators.
2. ML models classify pixels or 3D data to quantify plant features.

2. Predictive Modeling:

1. Predict yield, growth stage, or stress response using historical data.
2. ML models identify subtle patterns not visible to the human eye.

3. Genetic Analysis Integration:

1. Combine phenotypic and genotypic data.
2. AI helps identify genes controlling traits (GWAS, QTL prediction).

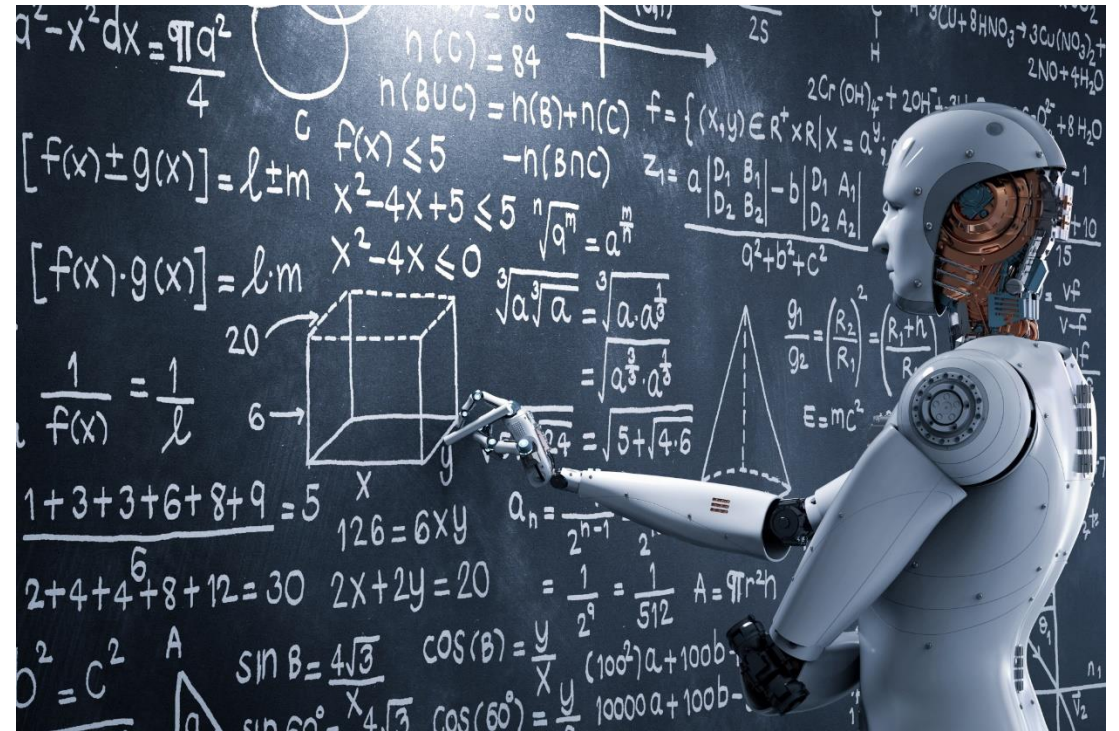
Notable studies in the area of artificial intelligence (AI) applications in high-throughput phenotyping (HTP)

Focus	Methodology	Key findings
Plant phenotyping with deep learning	Image analysis+CNN	Accurate leaf segmentation and trait quantification (Minervini et al. 2015)
Automated HTP	Hyperspectral imaging+machine learning	Rapid assessment of crop performance (Montes 2007)
Disease detection using AI	UAV imagery+deep learning	Early identification of diseases in wheat (Singh et al. 2021)
Genomic selection in plant breeding	Genomic data+machine learning	Enhanced prediction of breeding outcomes (Crossa et al. 2017)
Optimizing crop breeding with AI	Bayesian optimization+genomic prediction	Improved selection of high-yielding genotypes (Merrick et al. 2022)
Weed detection using computer vision	UAV imagery+Convolutional Neural Networks	Efficient identification of weed presence (Haq 2022)

Challenges in Lab HTPP – to work on...



- Data overload – big data problem
- Standardization across systems
- Lab-to-field translation gap
- Cost and accessibility





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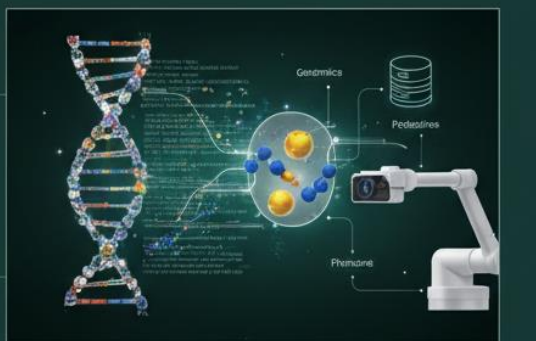
AI-driven trait prediction



Future Directions



Digital twins and crop models



Integration with omics data



Portable phenotyping solutions



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Thank you for your attention!

Any questions?

