



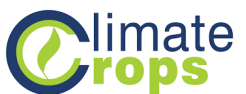
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Introduction to optics for plant breeders

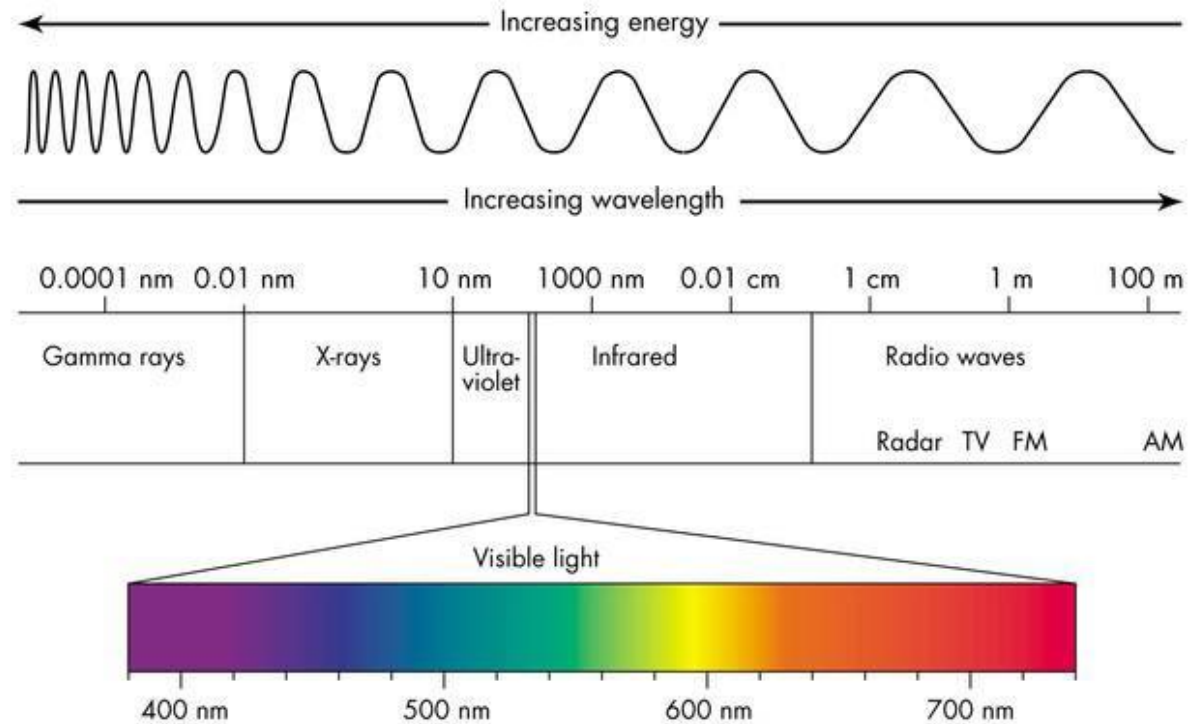
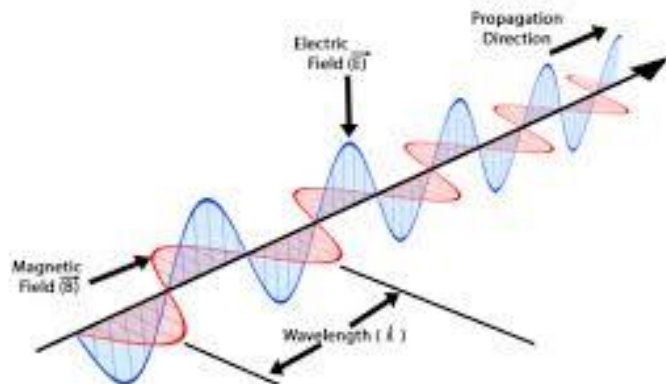
Dr. Vuk Đorđević

Legumes Department, IFVCNS

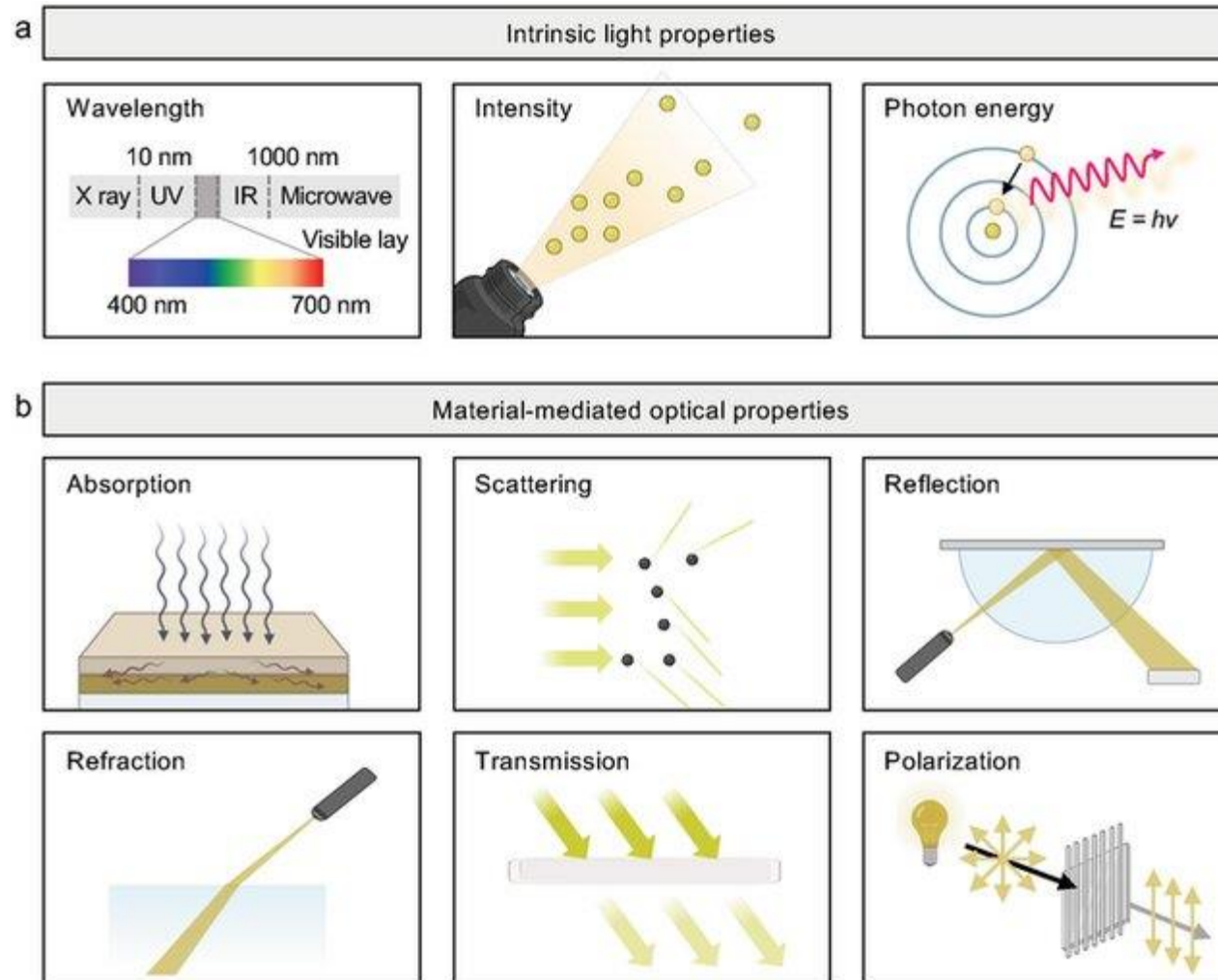


Nature of light

Electromagnetic Wave



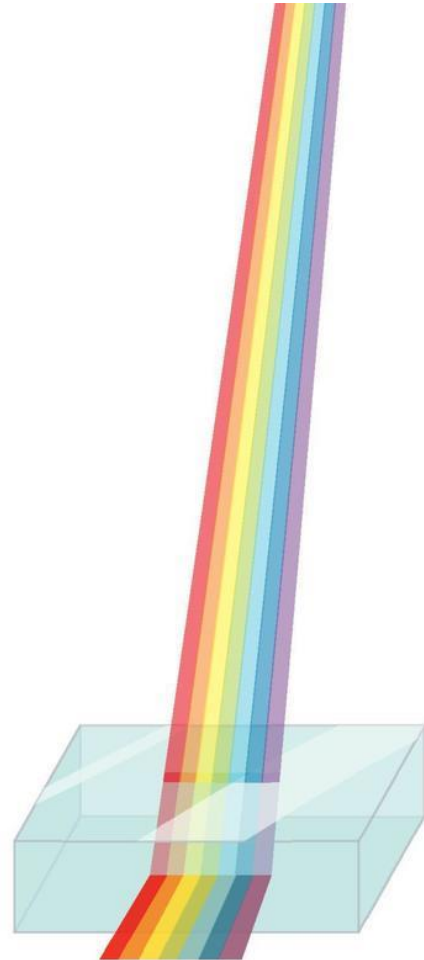
Light properties



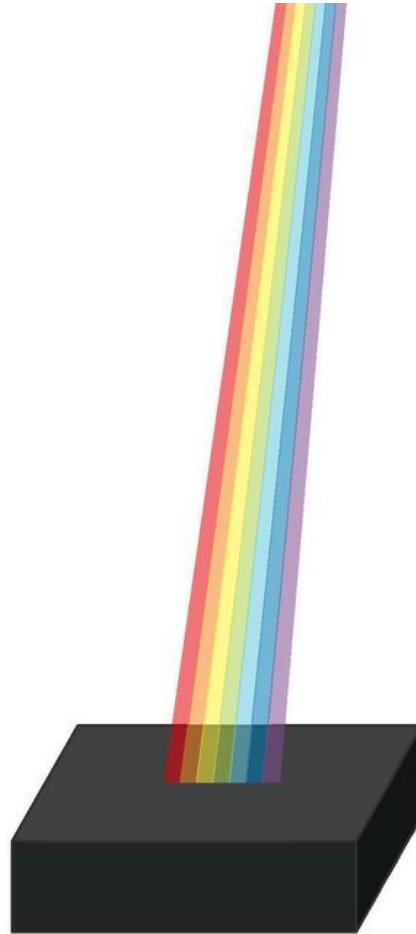


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Transmission



Absorption



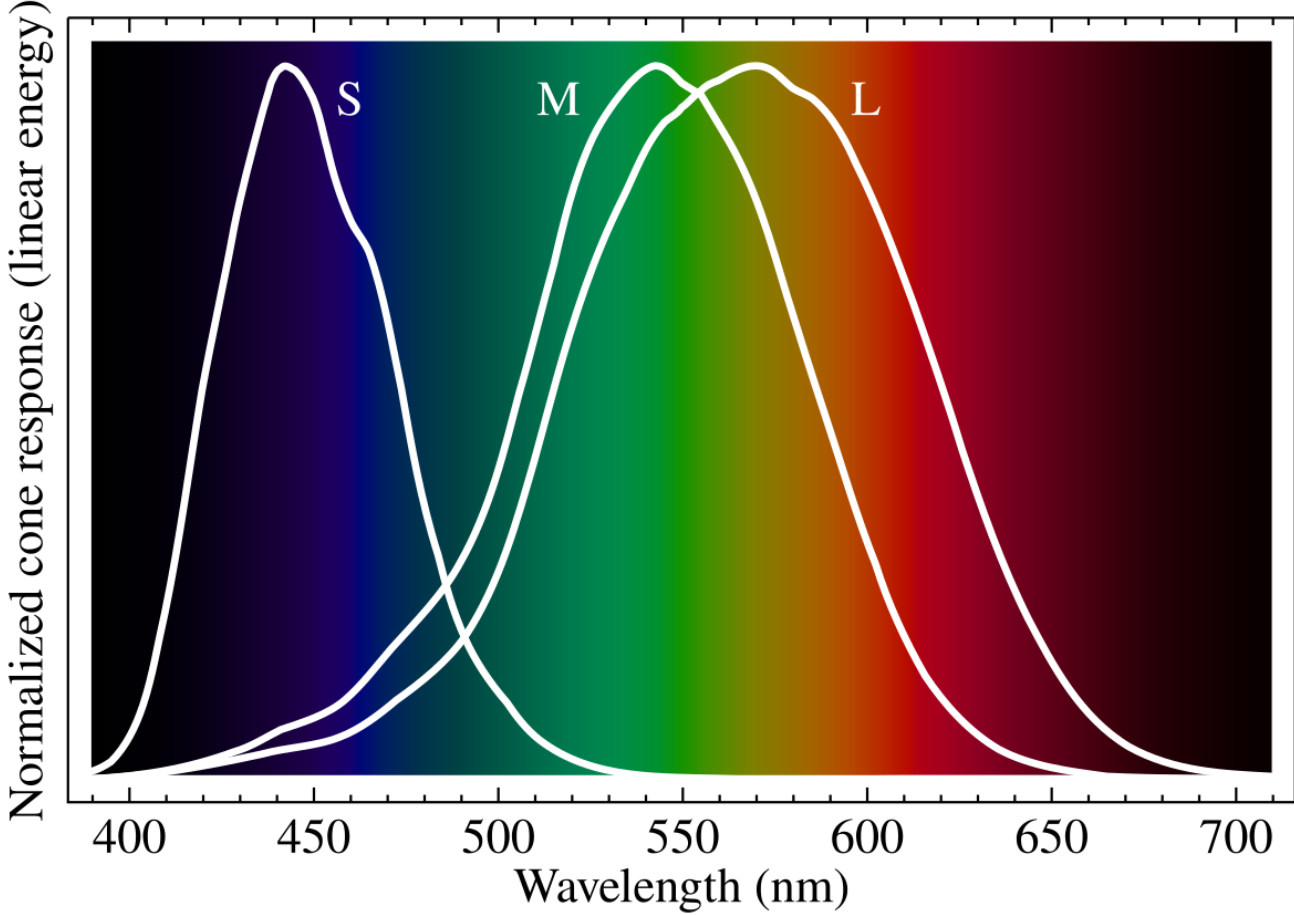
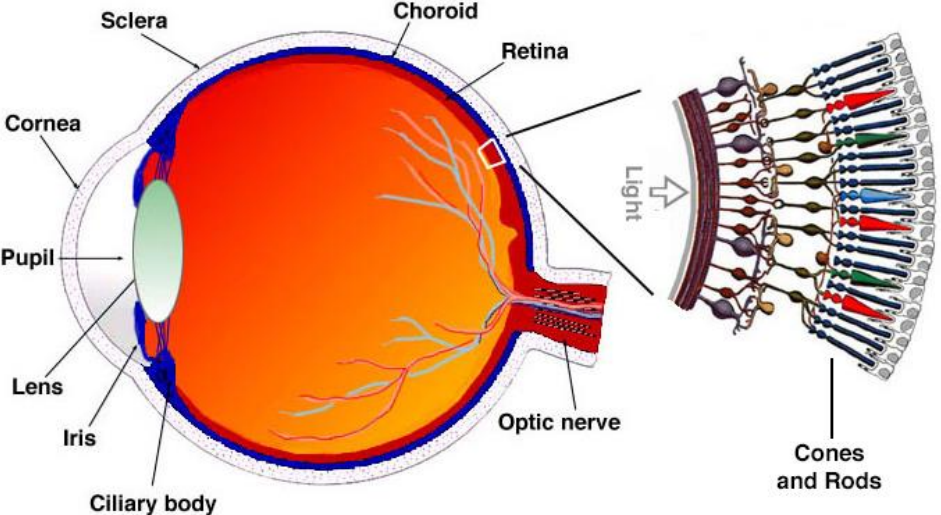
Reflection

properties of light



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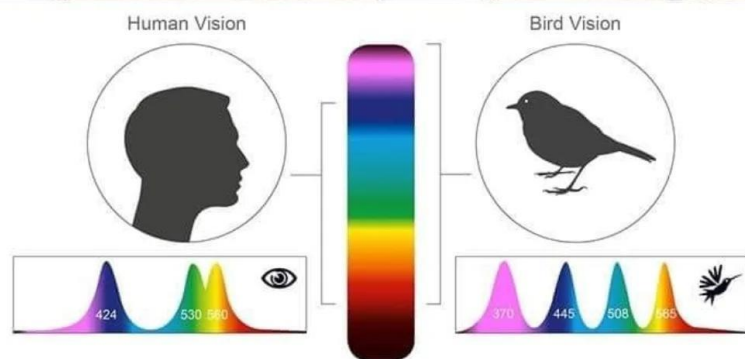
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Human View

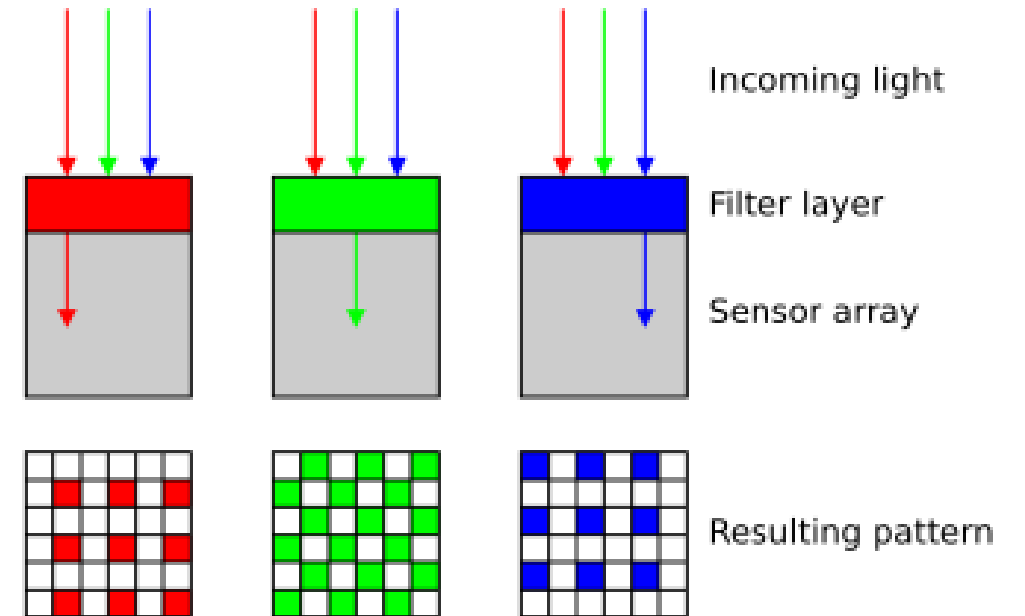
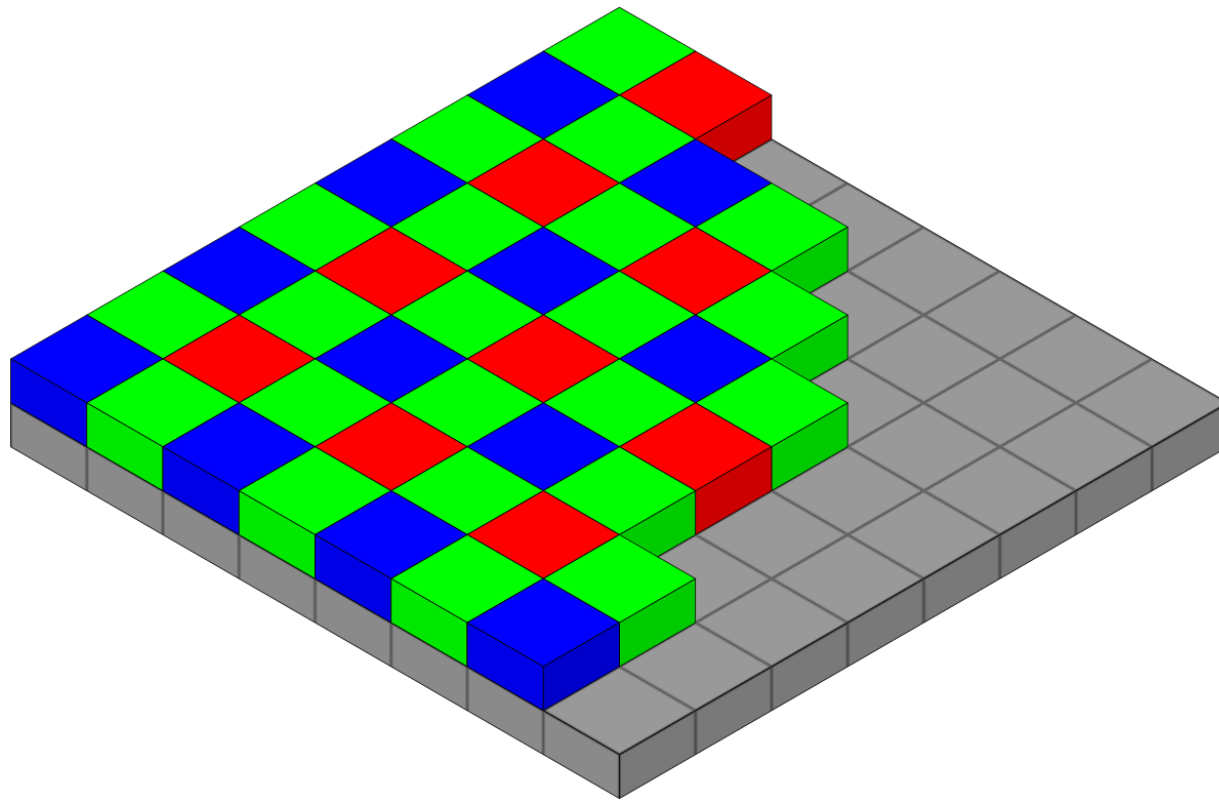
Dog View





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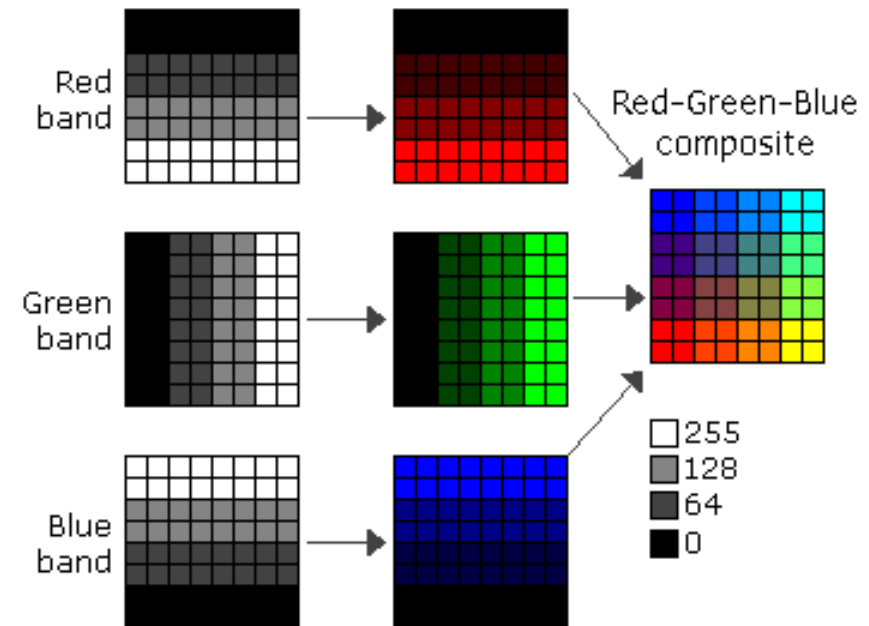
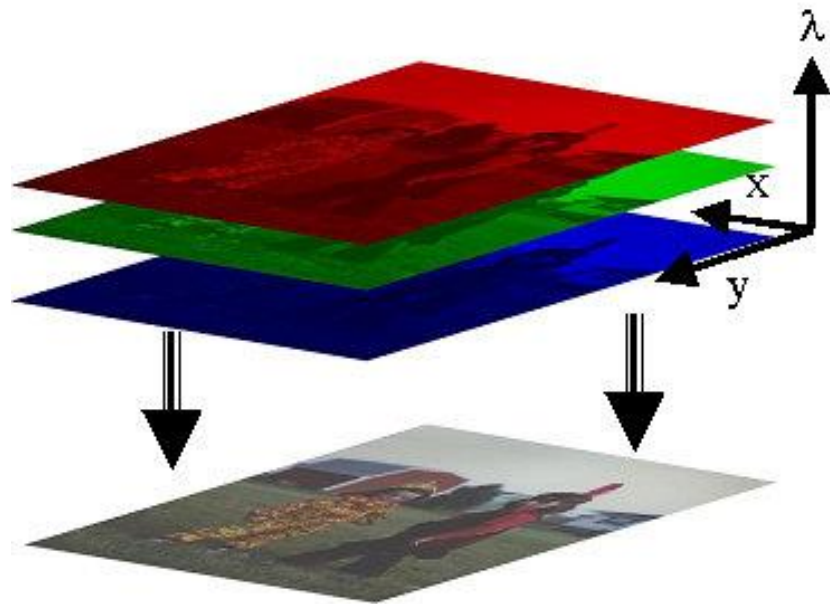
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HTPP in Field Trials

Dr. Predrag Randelović

Legumes Department, IFVCNS





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The Science of Phenotyping

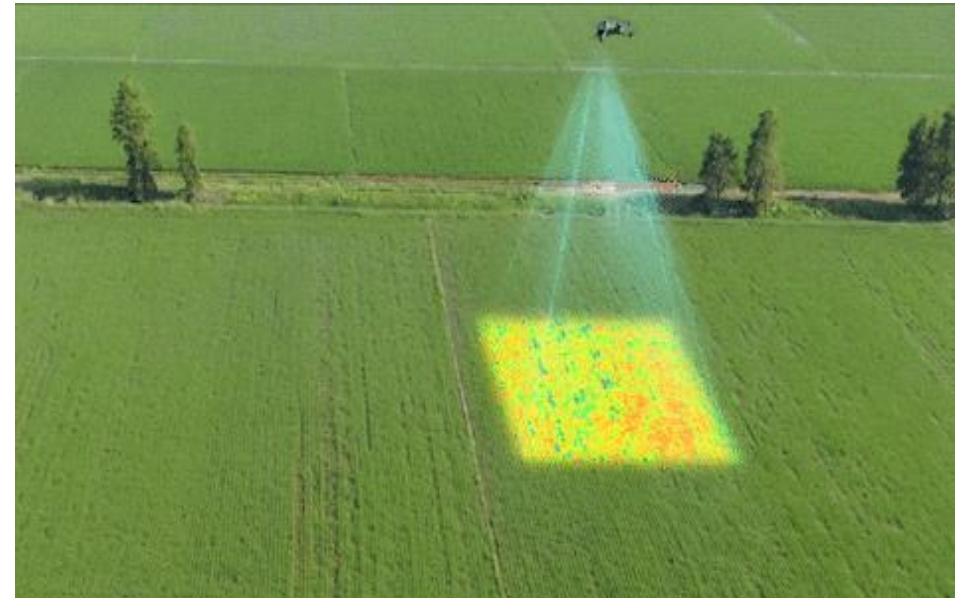
Phenotyping is the discipline of observing, measuring, and analyzing the phenotype resulting from genotype-environment interactions.

- ❑ **The Challenge:** Traditional phenotyping relies on manual measurements that limit our ability to analyze large plant populations efficiently.



What is HTPP?

- High-throughput phenotyping (**HTPP**) combines different platforms, digital imaging sensors, and advanced analytics
- Enables fast assess of various plant characteristics across large agricultural areas
- HTPP transforms agricultural monitoring from labor-intensive processes to automated, data-driven insights
- Can be integrated within breeding programs



Traditional VS. HTPP



Traditional Phenotyping

Advantages:

- Simplicity in implementation
- Minimal equipment requirements
- Lower initial investment costs

Disadvantages:

- Time-intensive processes
- High labor costs
- Greater potential for human error
- Limited data collection capacity

High-Throughput Phenotyping

Advantages:

- High efficiency and throughput
- Enhanced precision and accuracy
- Rapid response times
- Non-invasive measurement approaches
- Long-term cost effectiveness

Disadvantages:

- High initial equipment costs
- Requires specialized personnel
- Complex data processing requirements



The Foundation: Remote Sensing & Photogrammetry

01

Remote Detection Principle

Monitor changes in analyzed objects across space and time efficiently without direct contact

02

Light Reflection Analysis

Remote sensing collects data based on light reflection patterns from plant surfaces

03

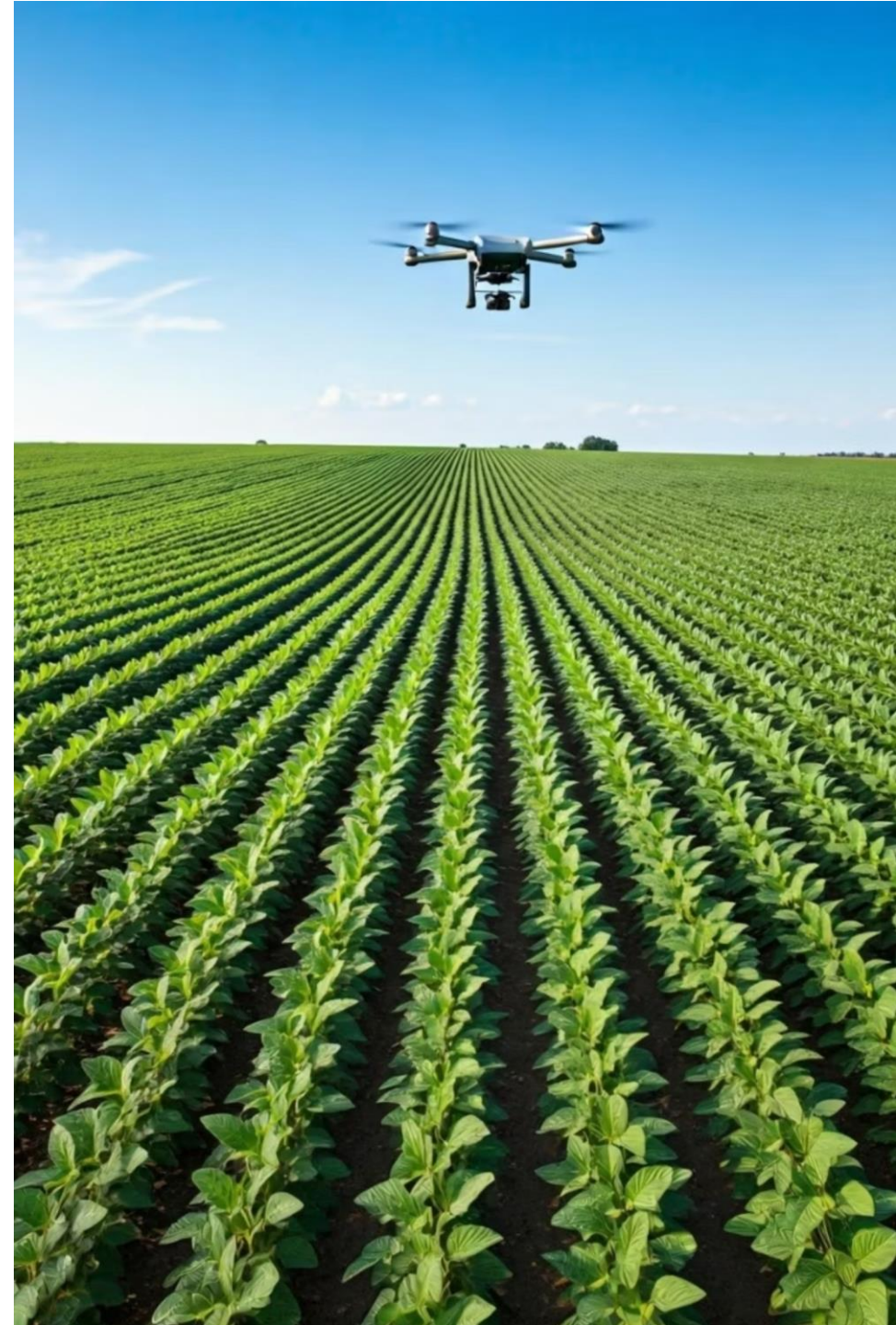
Photogrammetric Measurements

Photogrammetry enables precise measurements of observed objects from photographs

04

Three Platform Types

Satellites, ground-based equipment, and unmanned aerial vehicles (UAVs)





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Satellite Systems

Pros: Continuous coverage of large areas

Cons: Low resolution (0.3-20 m/pixel)

Best for: Large-scale monitoring



Ground-Based Equipment

Pros: High image resolution, hyperspectral imaging

Cons: Limited area coverage, high operational costs

Best for: Detailed plot analysis

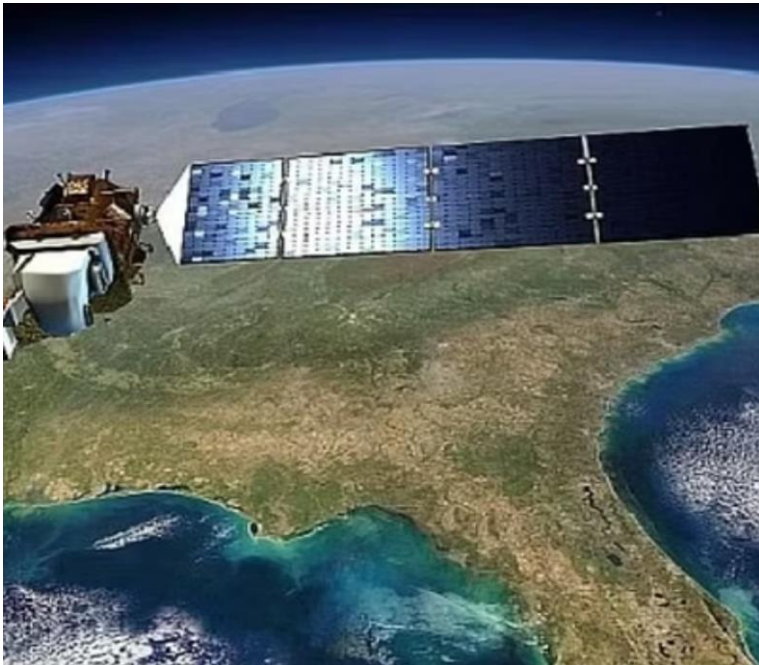


Unmanned Aerial Vehicles

Pros: Optimal balance of coverage and detail

Cons: Limited flight autonomy

Best for: Field trial phenotyping



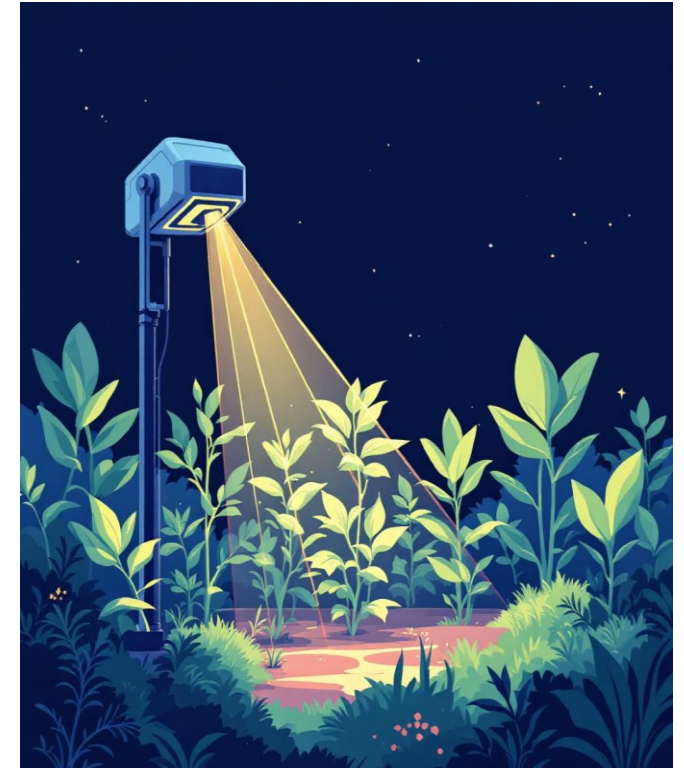
Active Sensors: LiDAR Technology

Operating Principle

Active sensors such as LiDAR (Light Detection and Ranging system), emit radiation and record values based on return signals.

Primary Applications

- Plant height determination with high precision
- Canopy structure analysis through vegetation penetration
- Biomass estimation and growth monitoring
- Crop density and distribution mapping



Current Limitations

High equipment costs, complex data acquisition processes, and specialized knowledge requirements for data processing remain significant barriers to widespread adoption.

Passive Sensor Technologies

Passive sensors detect reflected radiation from imaged objects and can function as standalone devices or integrated systems.

RGB Cameras (400-700nm)

Single optical sensor providing data from red, green, and blue spectral regions. Essential for visual assessment and basic morphological measurements.



Multispectral Cameras (400-1000nm)

Multiple monochromatic sensors covering R, G, B, Red Edge, and NIR channels. Ideal for vegetation indices health assessment, trait estimation.



Hyperspectral Cameras (400-1000nm)

Collect data across numerous wavelengths at nanometer intervals. Provide detailed spectral signatures for crop analysis.



Thermal Cameras

Determine temperature of imaged objects, primarily used for water stress detection and plant physiological monitoring.





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HTPP - Image Processing Workflow



Software Platform

Stitching software (Agisoft, Pix4D...) are used for comprehensive photograph processing and analysis



Dense Point Cloud (DPC)

Structure-from-Motion creates 3D models from multiple viewing angles



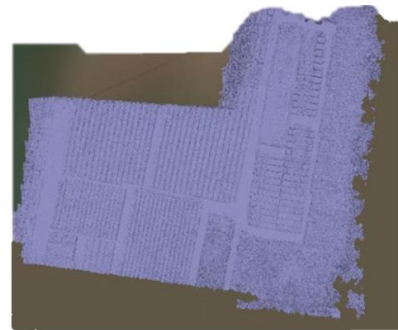
Spatial Alignment

Photographs aligned using common reference points for accurate positioning

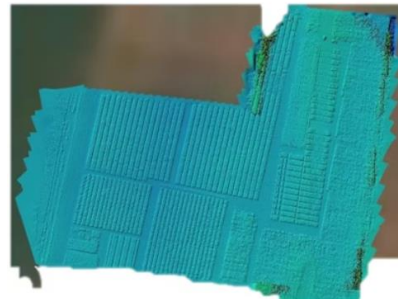


Final Products

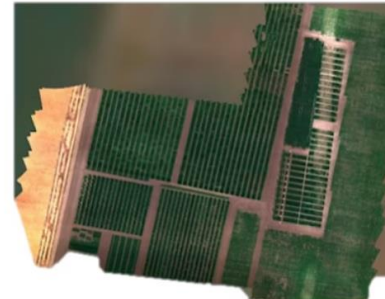
Digital Elevation Model (DEM) and orthomosaic generation for analysis



DPC



DEM



ORTHOMOSAIC

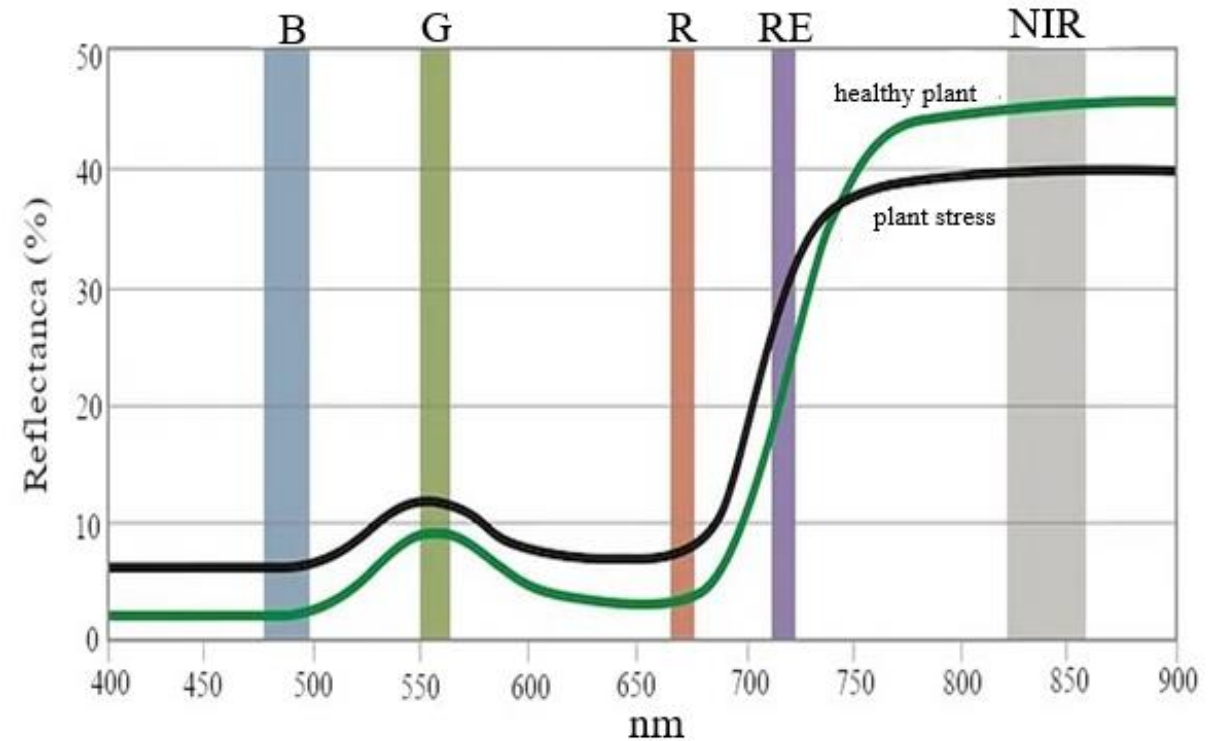
Spectral Reflectance Fundamentals

Healthy Plant Response

- Absorbs visible light efficiently
- Reflects NIR radiation strongly
- Optimal photosynthetic activity

Stressed Plant Response

- Increased RGB reflection
- Reduced NIR reflection
- Compromised chlorophyll function



Solar radiation reflection patterns depend on leaf chemical composition and physiological characteristics - enabling vegetation index calculations through mathematical transformations



Vegetation Indices Overview



RGB Indices

Combine visible spectrum data for segmentation, plant pixel detection, biomass assessment, and yield estimation



Multispectral Indices

Incorporate visible, red-edge, and NIR data for enhanced remote sensing applications

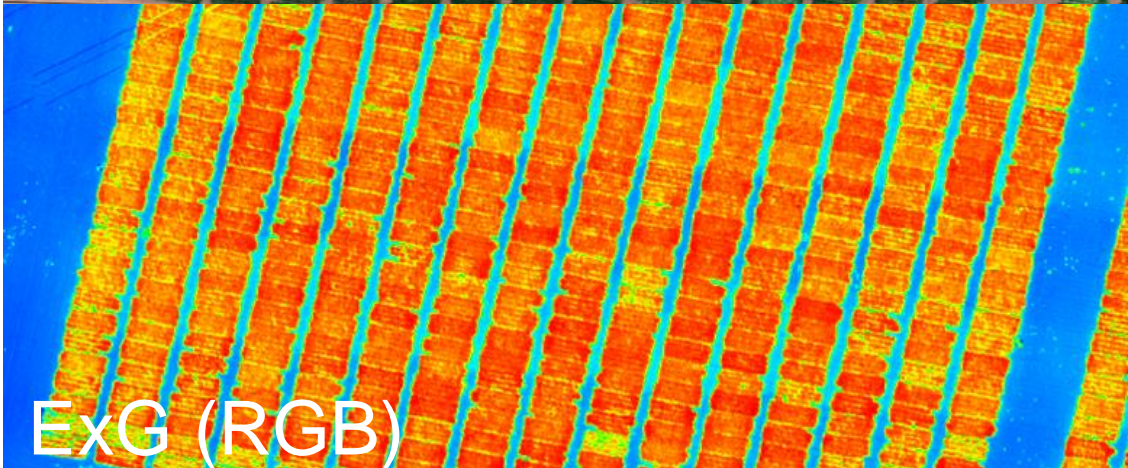


NDVI

Most recognized vegetation index combining NIR and red channels for crop health analysis, yield prediction, and nitrogen use efficiency



Orthomosaic



ExG (RGB)



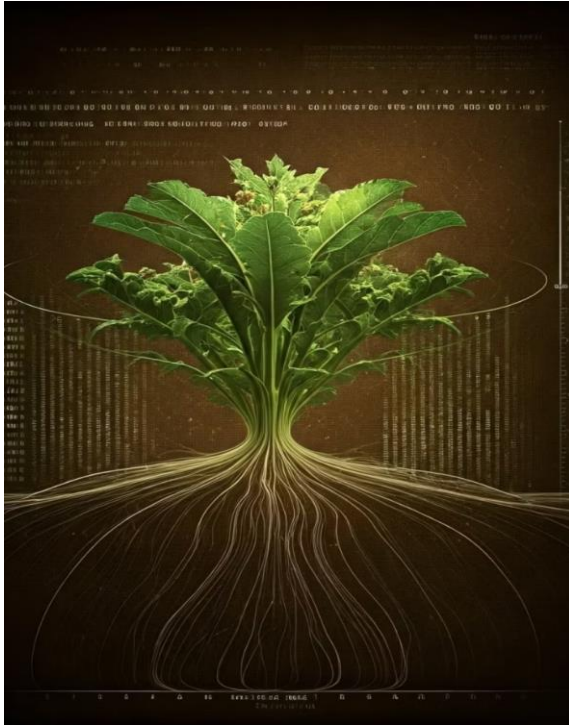
NDVI (multispectral)



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Machine Learning (ML) Integration



ML Algorithms

SVR, KNN, ANN, CNN, PLSR, RF enable pattern recognition in large datasets



HTPP and ML

Reliable prediction models



Cross Validation

Data splitting (training/test) reduces model overfitting risks

Random Forest (RF)

- Groups data using binary trees
- Effective for classification and regression
- Handles complex agricultural datasets

Partial Least Squares Regression (PLSR)

- Determines relationships through principal components
- Links dependent and independent variables
- Reduces dimensionality effectively



HPP application

01

Quantitative Trait Assessment

Evaluation of multiple traits critical for new genotype selection

02

Growth Monitoring

Dynamic tracking of plant development throughout growing season

03

Stress Quantification

Measurement of abiotic and biotic stress factors affecting performance

04

Enhanced Breeding Efficiency

Improved germplasm characterization through precise phenotypic analysis





HTPP applications - plant height

01

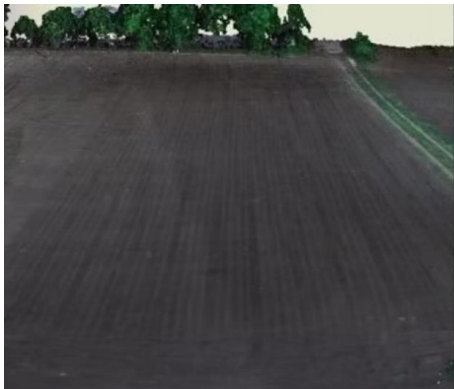
DSM captures crop canopy surface

Digital Surface Model records top elevation of vegetation and terrain

03

Plant Height = DSM – DTM

Precise phenotypic measurements for breeding programs

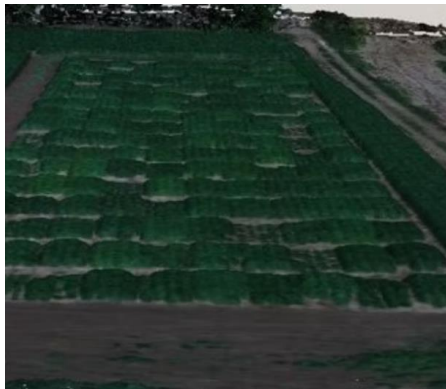


DTM - Digital Terrain Model

02

DTM represents bare soil baseline

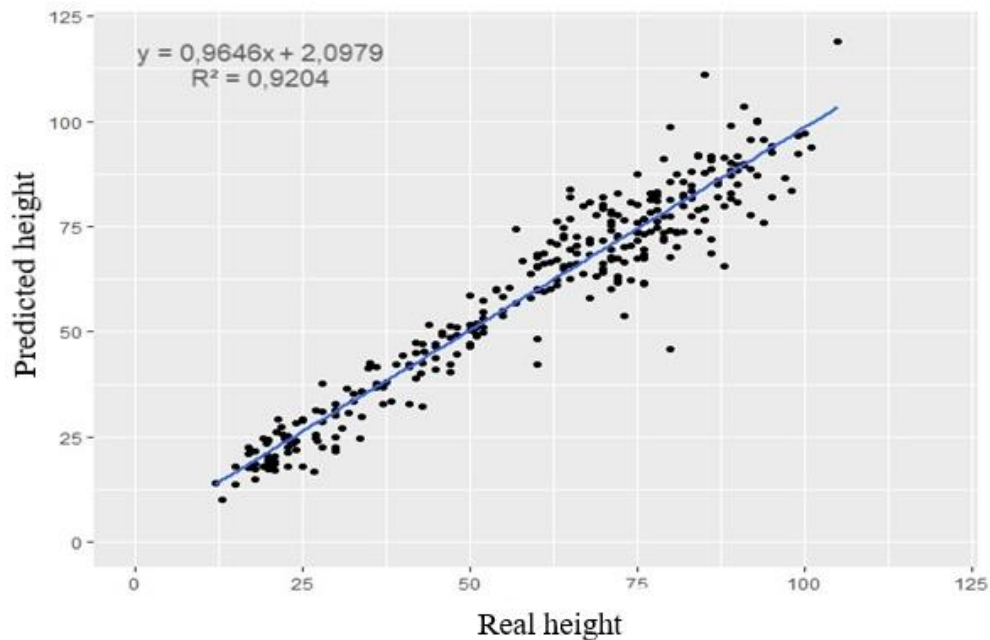
Digital Terrain Model provides ground reference elevation




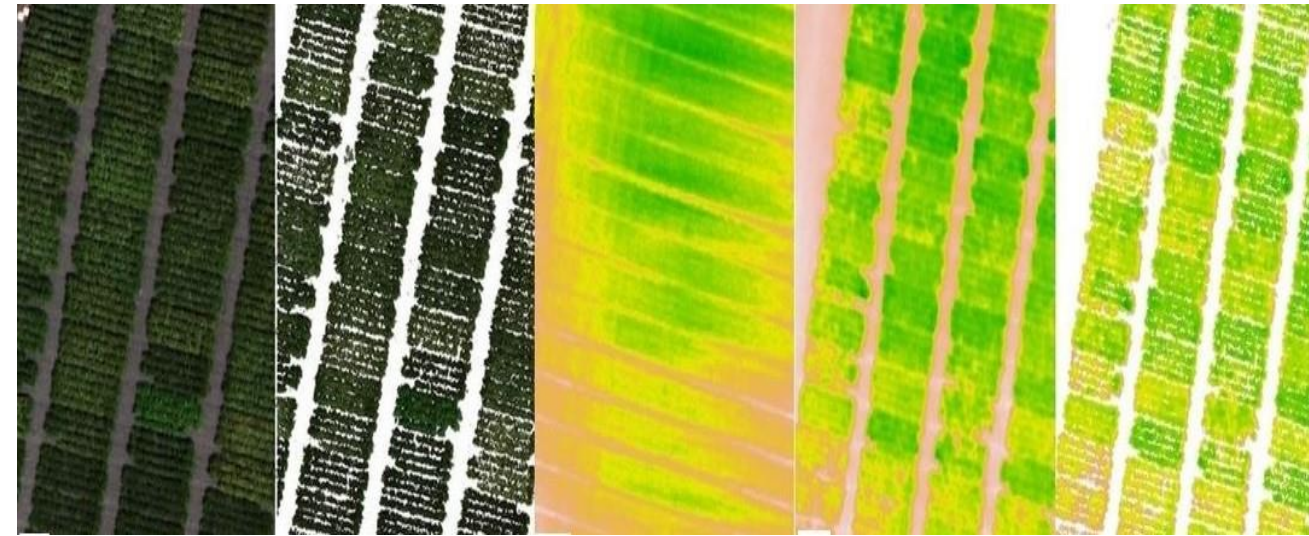
DSM - Digital Surface Model



HTPP applications - plant height



 **Strong correlation** between ground-measured and HTPP-derived plant heights validates remote sensing accuracy



Orthomosaic

Mask

DTM

DSM

PH

1

Orthomosaic

High-resolution aerial imagery

2

Plot Mask

ROI boundary definition

3

DTM Generation

Terrain elevation baseline

4

DSM Creation

Surface height mapping

5

PH Calculation

Final height measurement



HTPP applications - canopy cover (CC)

01

Plot Identification

ROI creation for each experimental plot with precise boundaries

02

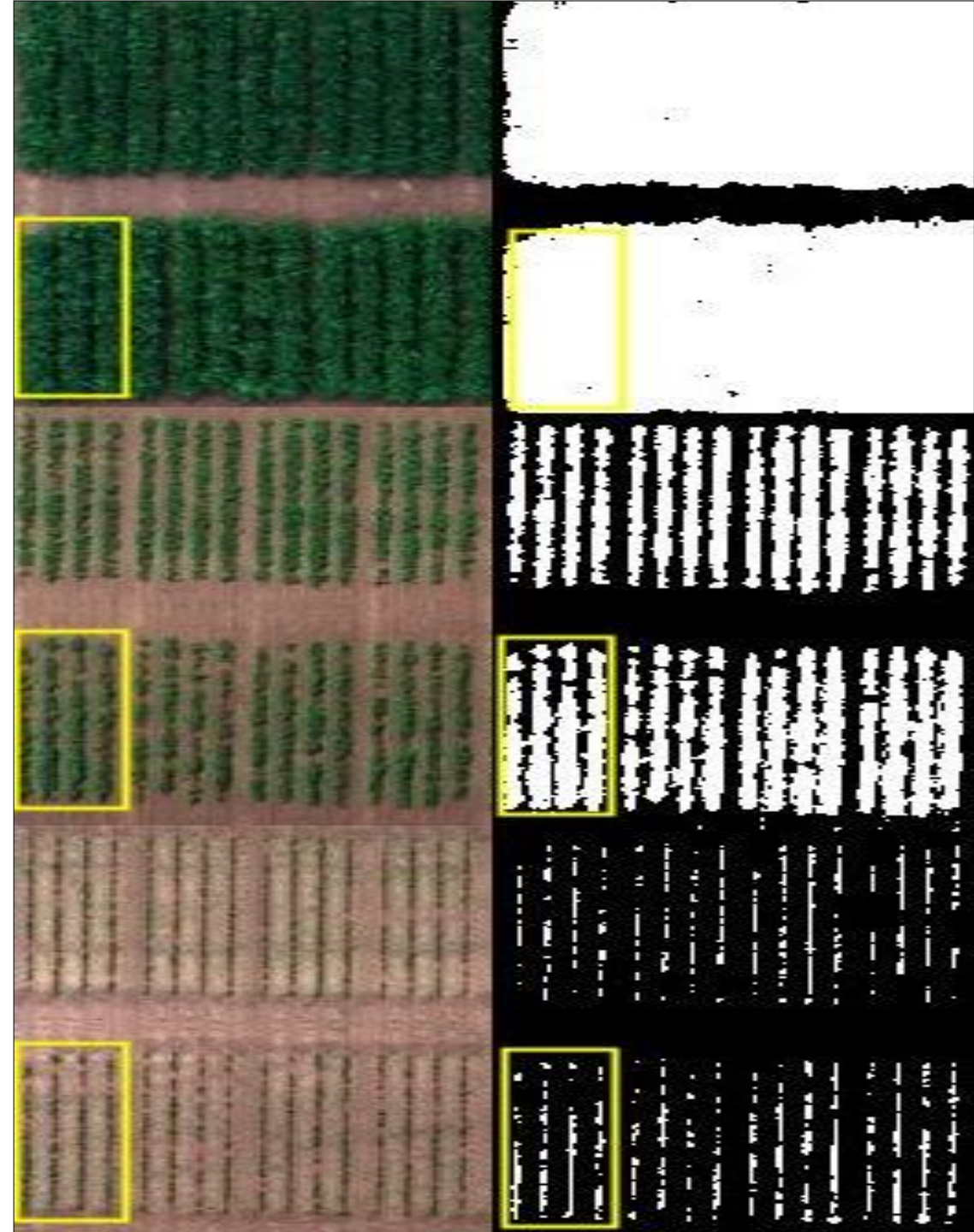
Spectral Analysis

NDVI and other vegetation indices for plant/soil
segmentation

03

CC estimation

Binary image analysis determines percentage of plant pixels within each
ROI



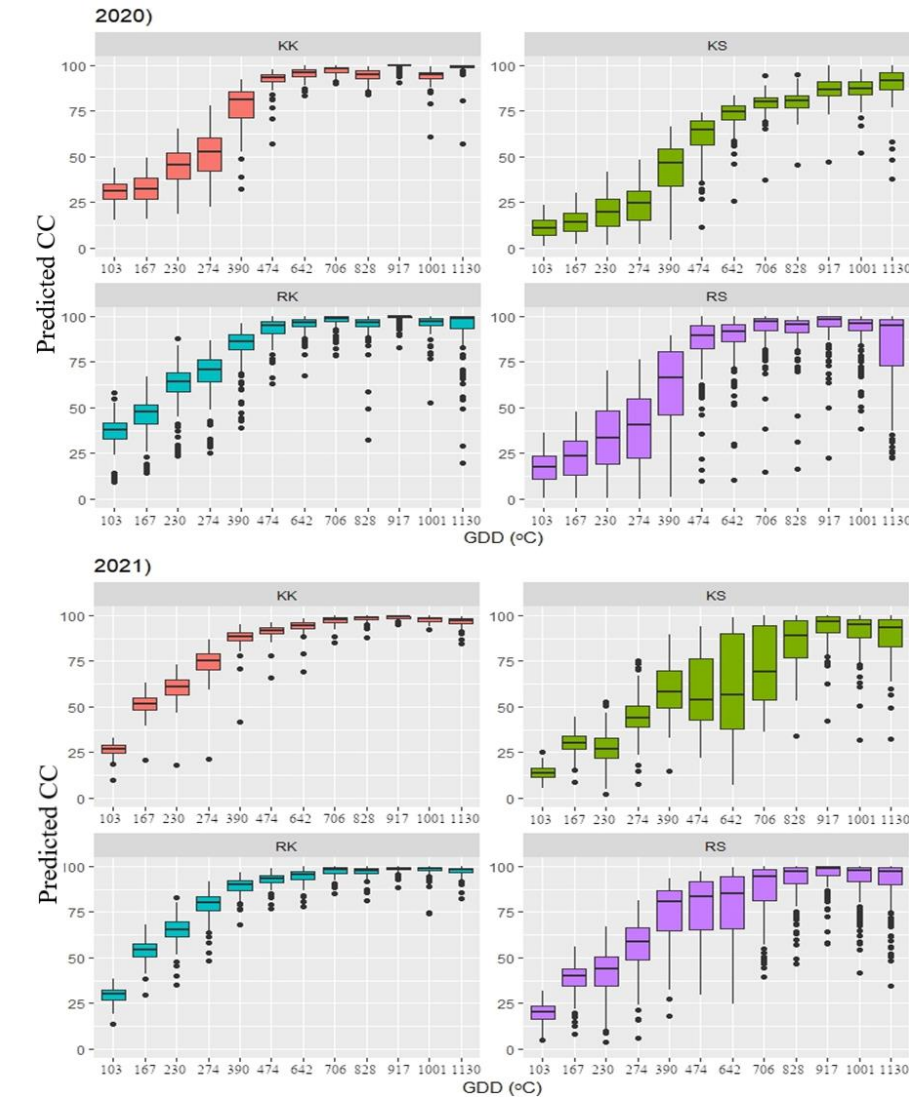


Canopy Cover Dynamics

- **Seasonal dynamics:** Track canopy cover development throughout the growing season
- **CCmax analysis:** Quantify days with >90% canopy coverage for genotype comparison
- **Selection criteria:** Identify genotypes with rapid canopy closure for improved resource utilization



This temporal analysis enables breeders to select for early vigor and efficient light interception traits.



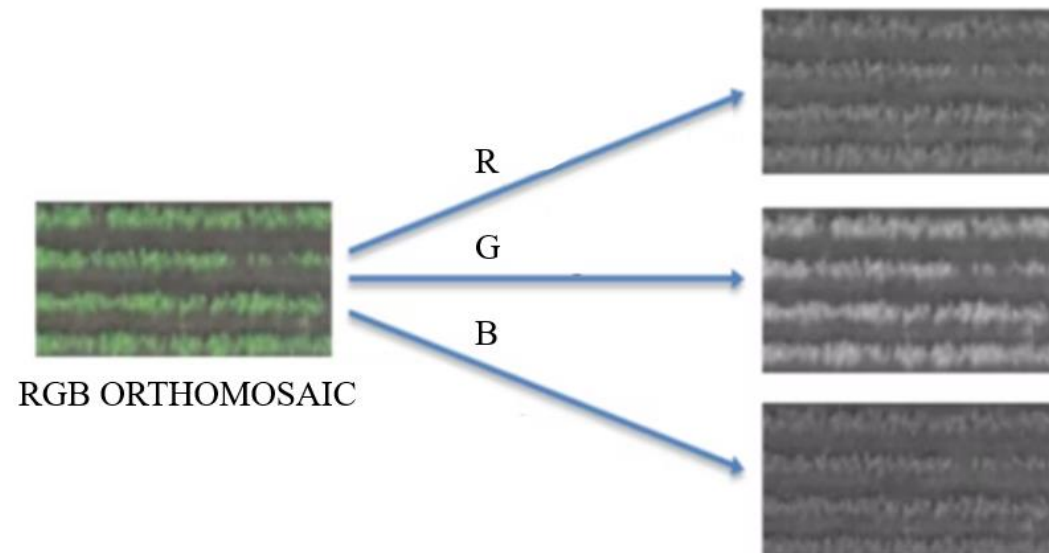
HTPP applications - plant density



1

Channel Separation

RGB channels extracted from orthomosaic images for individual spectral analysis



2

Vegetation Index Calculation

Multiple vegetation indices computed for each experimental plot



3

Random Forest Modeling

Algorithm uses vegetation indices as predictors for density estimation



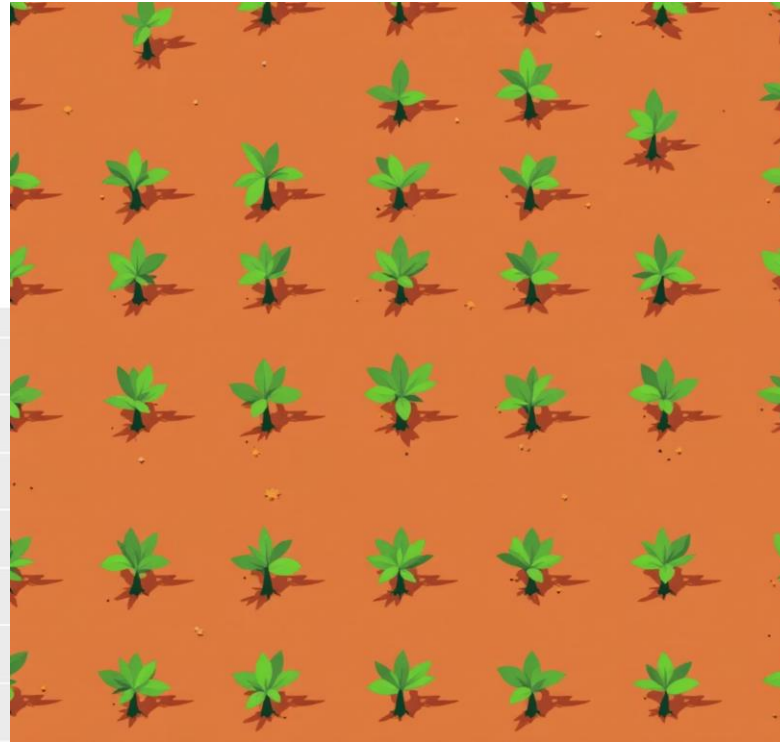
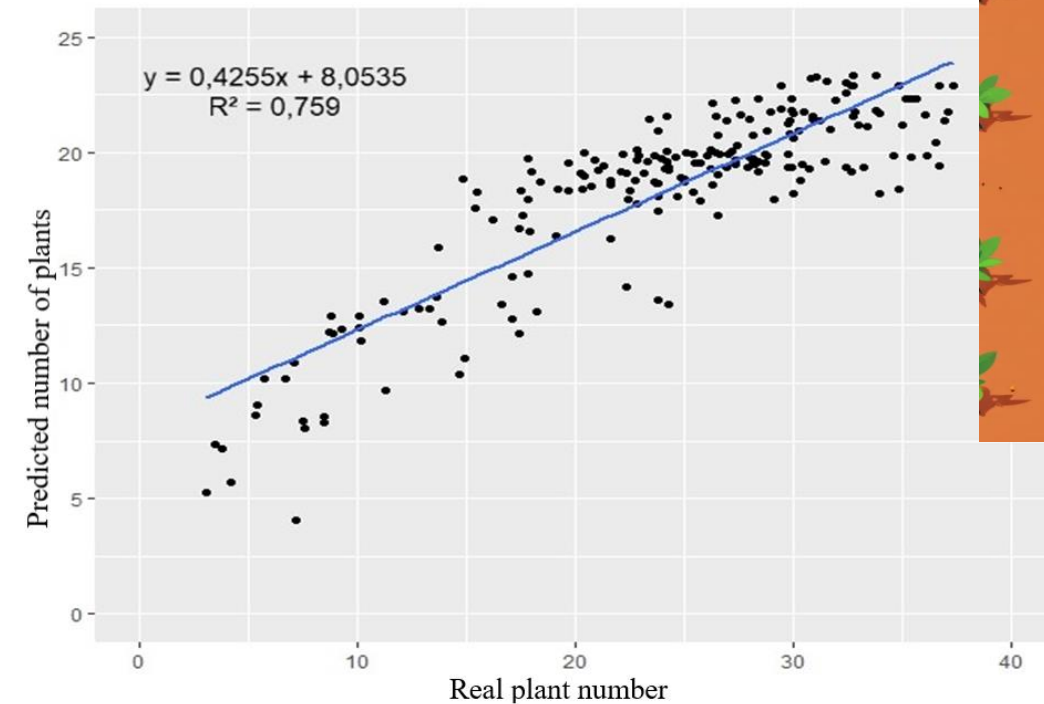
4

Model Validation

Performance assessed using correlation, R^2 , RMSE, and MAE metrics

Vegetation index	Name	Formula
TGI	Triangular greenness index	$G - 0.39R - 0.61B^*$
GLI	Green leaf index	$(2G - R - B) / (2G + R + B)$
NG	Normalized green	$G / (R + G + B)^*$
ExGR	Excess green red	$(3G - 2.4R - B) / (R + G + B)^*$
RGD	Red green difference	$R - G$
NGRD	Normalized green red difference	$(G - R) / (G + R)$
MNGRD	Modified normalized green red difference	$(G^2 - R^2) / (G^2 + R^2)$
MExG	Modified excess green	$1.262G - 0.884R - 0.311B$

HTPP applications - plant density



- **Emergence monitoring:** Early-season assessment of germination rate and row establishment
- **In-season monitoring:** Detection of plant loss due to environmental stress or disease
- **Final plant count:** Accurate harvest population for yield component analysis

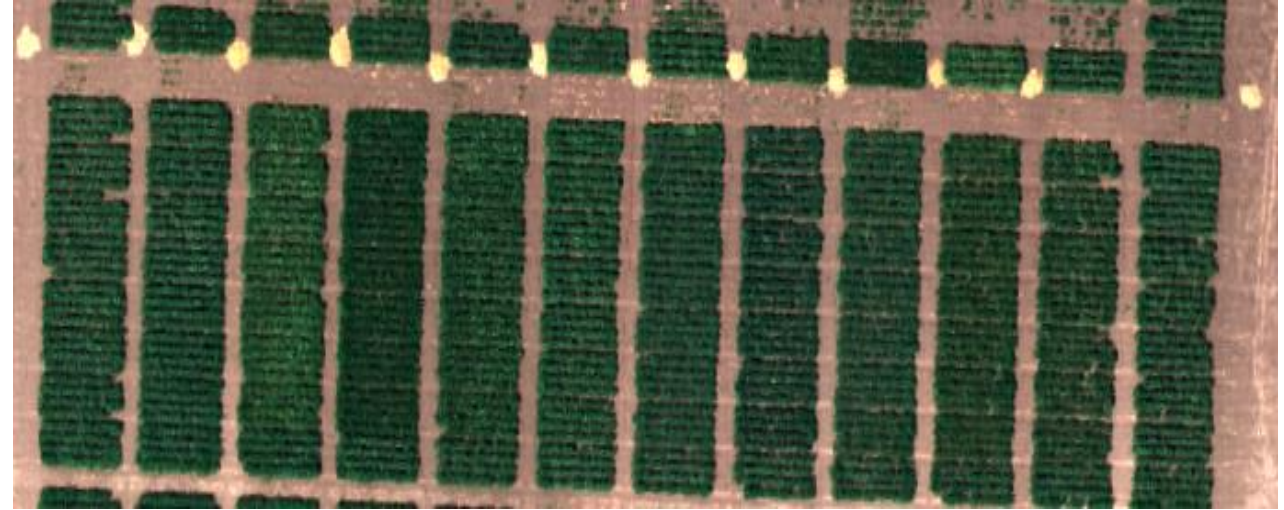
These applications provide comprehensive plant population data throughout the growing season, enabling precise agronomic management and breeding decisions.

HTPP applications - biomass estimation

Data Collection

1

Extraction of vegetation indices, structural metrics (height, volume), and texture analysis from high-resolution UAV imagery



Predictive Modeling

2

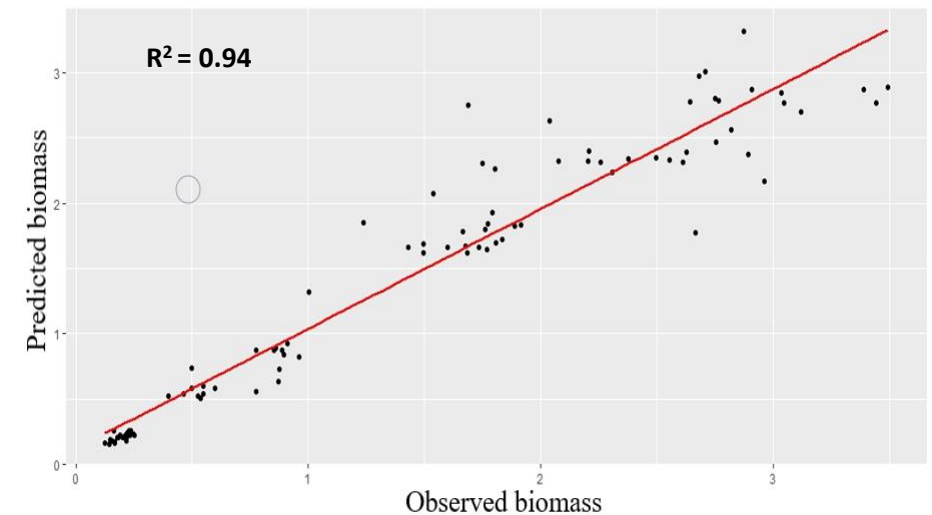
Machine learning algorithms process spectral, structural, and textural features to create robust biomass prediction models



Key Finding

3

Plant height emerges as most critical variable for biomass prediction accuracy





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HTPP applications - biomass estimation

Resource Utilization Assessment

Rating plant potential to use available resources efficiently

Growth Monitoring

Biomass as crop development indicator throughout season

Organic Matter Dynamics

Accumulation patterns for yield prediction modeling





HTPP Implementation Challenges



Plant Architecture Complexity

Plant architecture and dense planting lead to early plant overlap, complicating individual plant detection



Growth Stage Selection

Proper timing of image acquisition is critical for creating accurate predictive models and measurements



Weed Interference

Weeds increase plant pixel count and negatively impact model performance and phenotyping accuracy



Data Interpretation

Biological interpretation of HTPP data remains the primary challenge for practical application



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Key Takeaway

Transforming Plant Breeding with HTPP & AI

Rapid Data Assessment

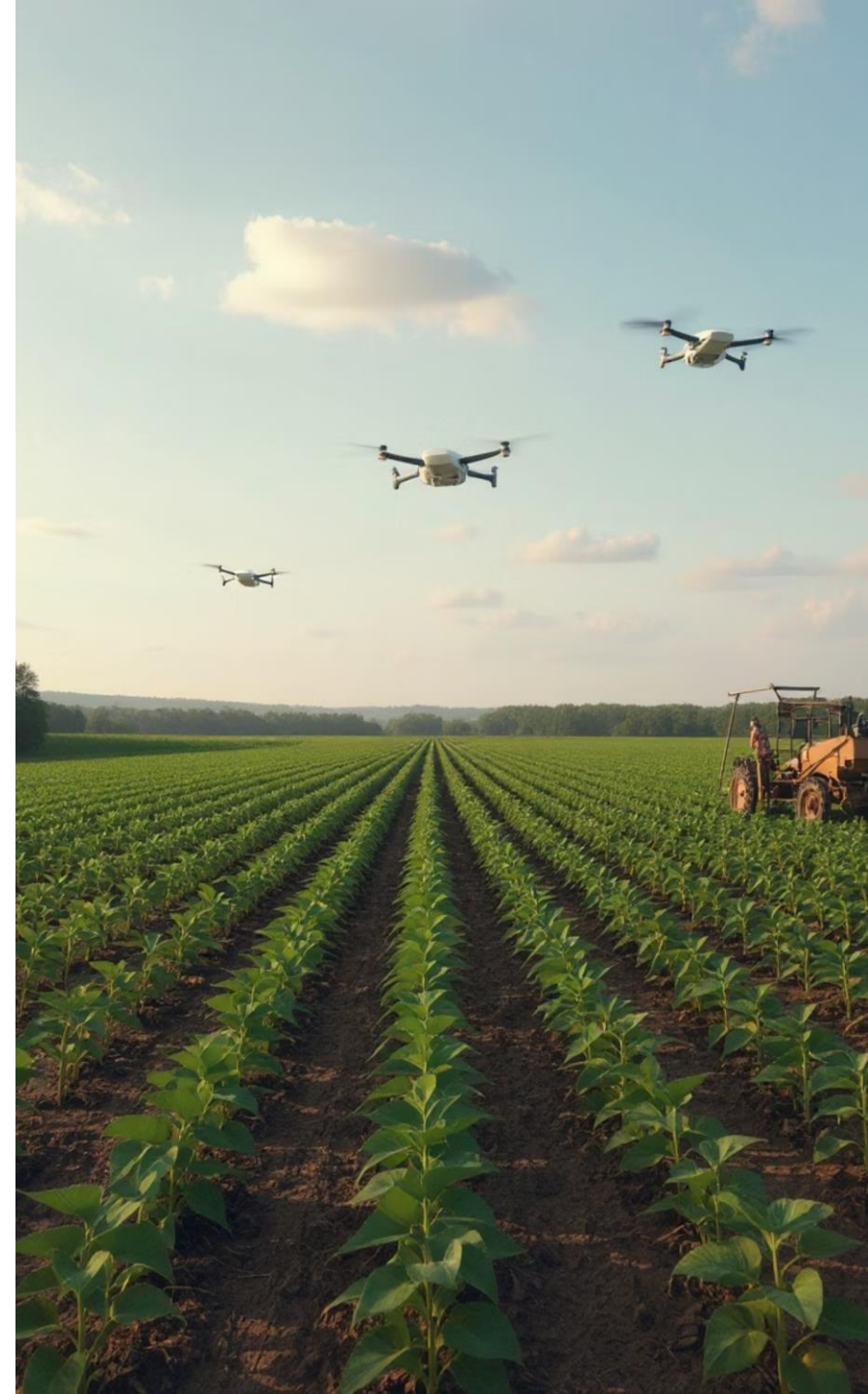
HTPP enables **fast, non-invasive assessment** of plant traits across large populations

AI-Powered Insights

Artificial Intelligence and Machine Learning unlock the full potential of HTPP data

Breeding Innovation

Faster, more accurate decisions in selection of superior varieties.





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Thank you for your attention!

Any questions?

