



High Throughput-Phenotyping at CIMMYT: Experiences and needs

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2021 R. F. Baker Plant Breeding Symposium
Predicting in the Future of Plant Breeding

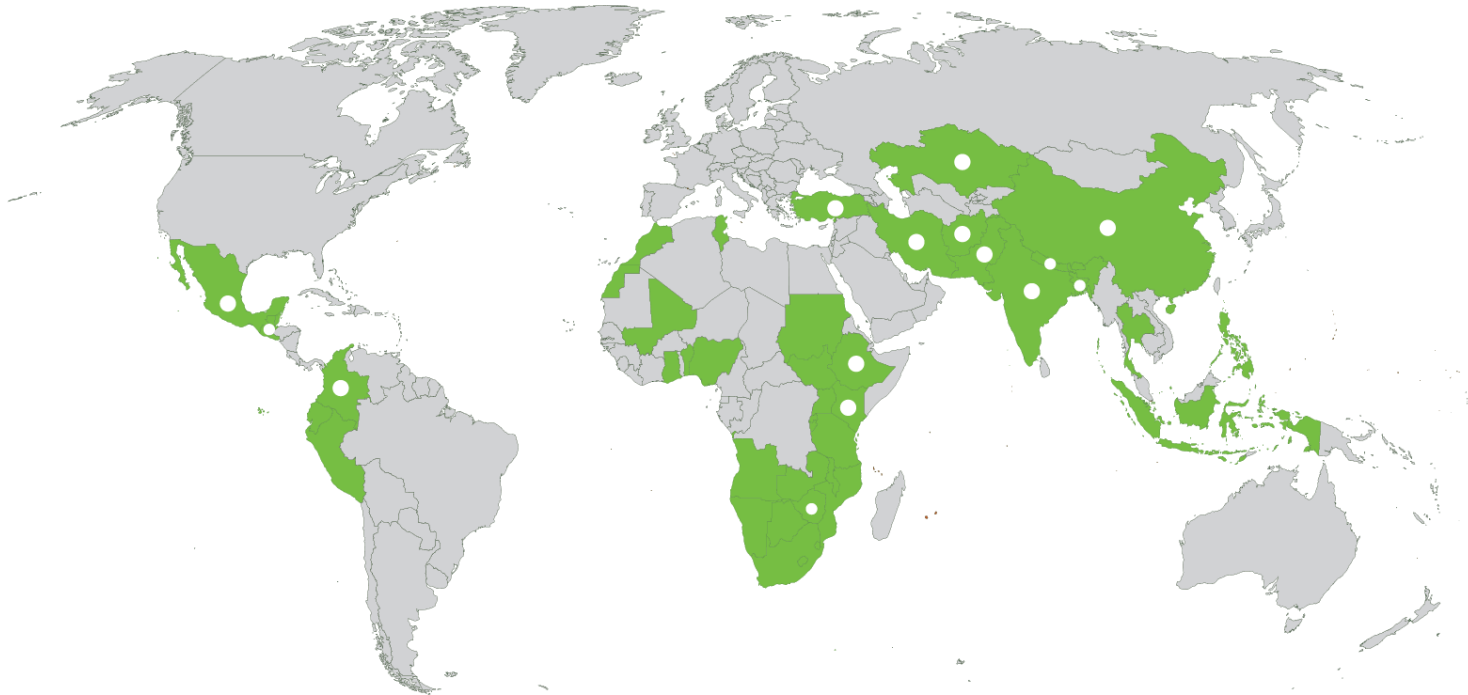
CIMMYT around the world

1,200 staff from over 50 countries!

CIMMYT works mainly in the developing world, aiming at improving livelihood by promoting more productive and sustainable maize and wheat agrosystems.



Dr. Norman Borlaug



Countries with offices:

Afghanistan
Bangladesh
China
Colombia
Ethiopia
Guatemala
India
Iran
Kazakhstan
Kenya
Mexico
Nepal
Pakistan
Turkey
Zimbabwe

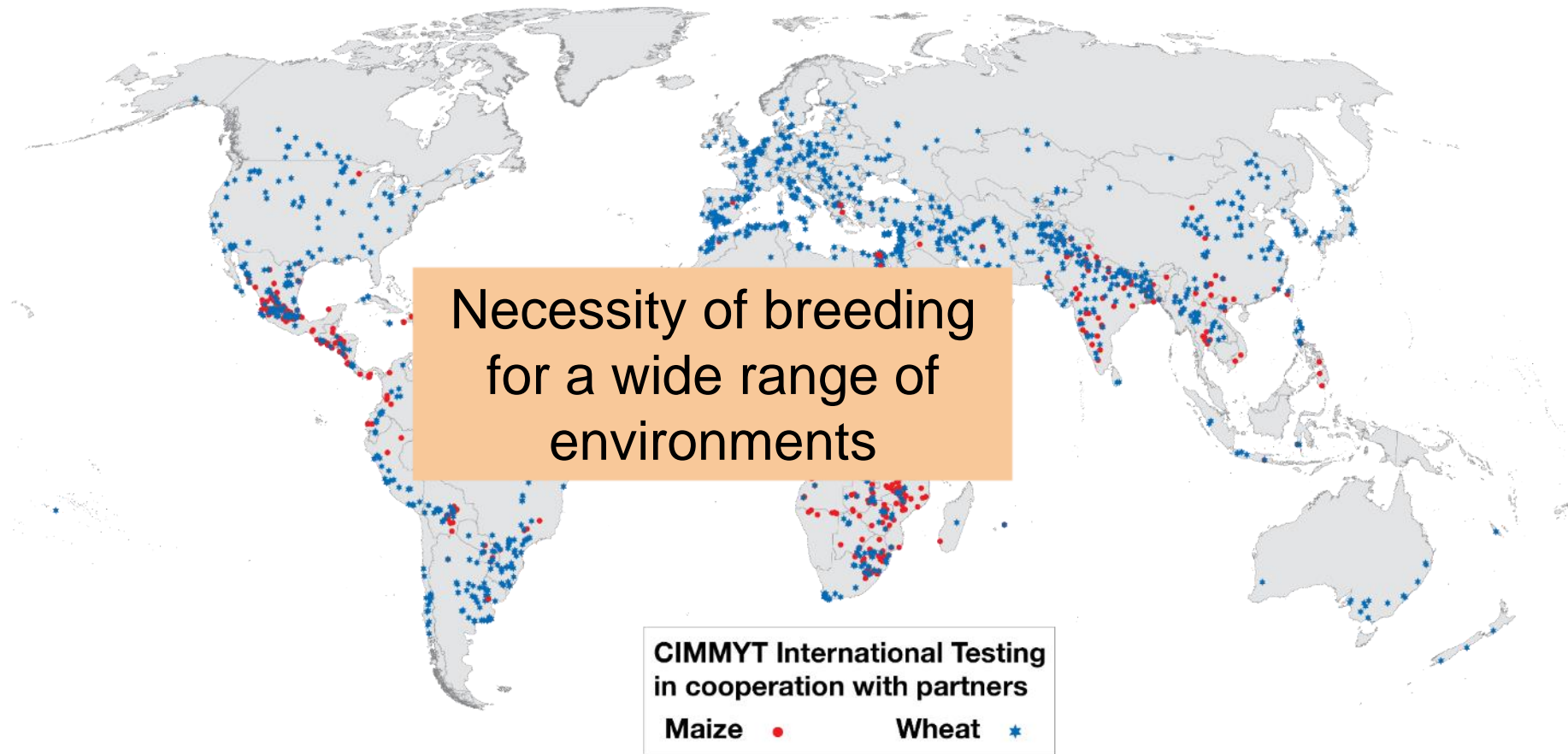


Consultative Groups for
International Agricultural
Research

Projects in over 40 countries



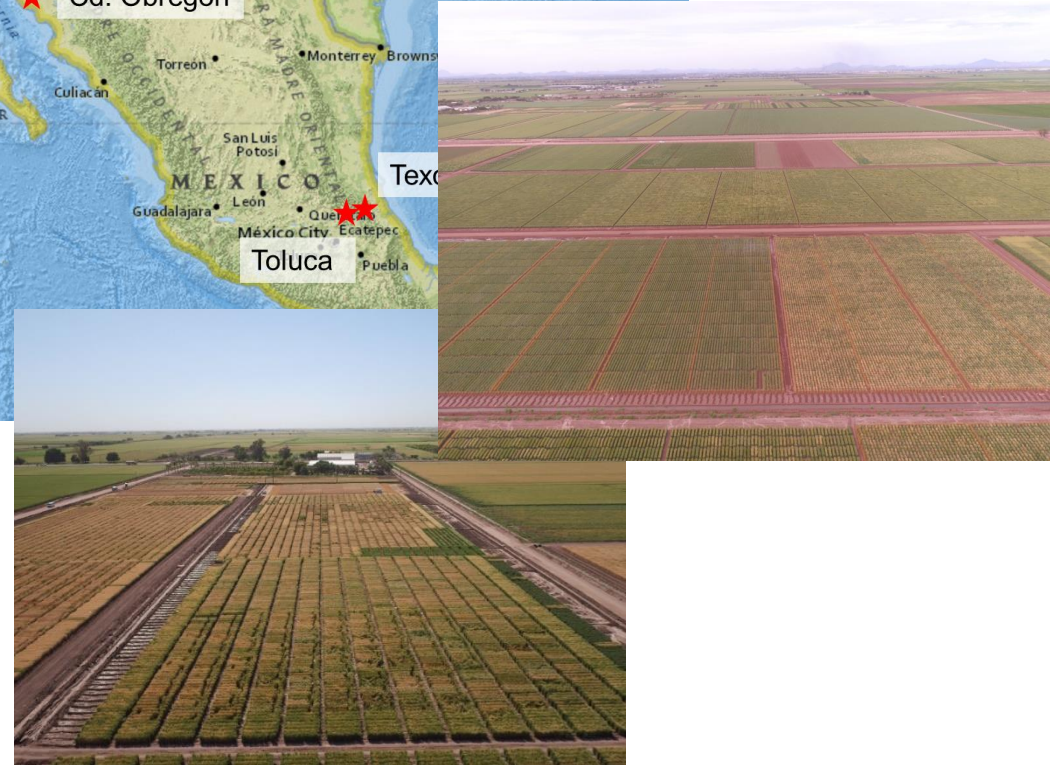
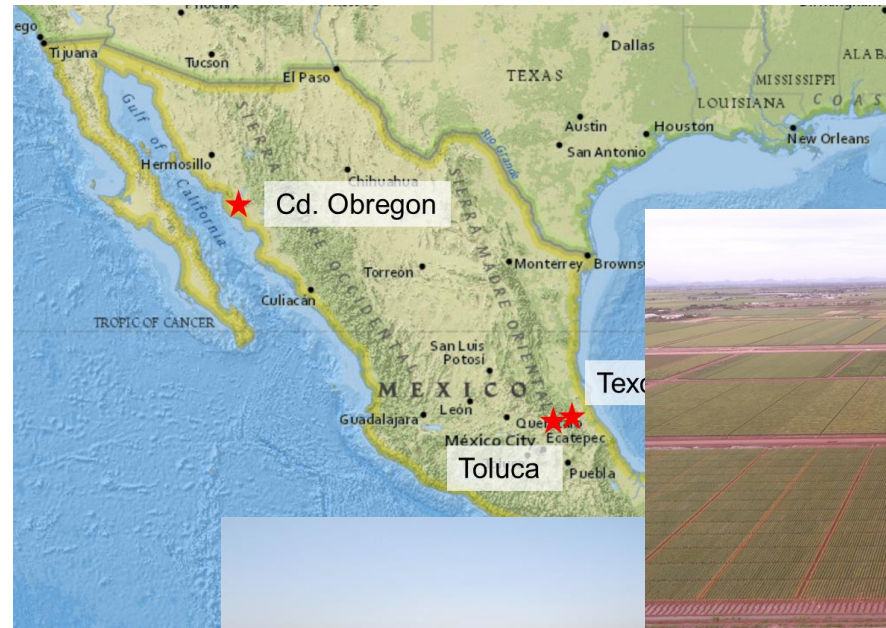
Global seed distribution network



50% of maize and wheat in the developing world is
based on CIMMYT varieties

Where everything starts: the experimental sites of the Global Wheat Program

- Cd. Obregon, Toluca, Texcoco:
 - Breeding, pre-breeding and research for different environments
 - International research collaborations
 - >100,000 plots (>250 Ha)
 - Phenotyping different traits for:
 - Early selection
 - Disease resistance evaluation
 - Physiology research
 - Physiological breeding
 - Exploration of genetic resources
 - Progeny evaluation



Plant phenotyping: a cornerstone in breeding programs

Plant phenotyping: quantitative description of plant's anatomical, ontogenetical, physiological and biochemical properties

Walter et al. 2015, Plant Methods

Agronomic traits:

- Yield
- TKW
- Phenology
- Plant height
- Disease resistance
- Biomass



High-throughput
phenotyping



Genomic selection models



Pedigree

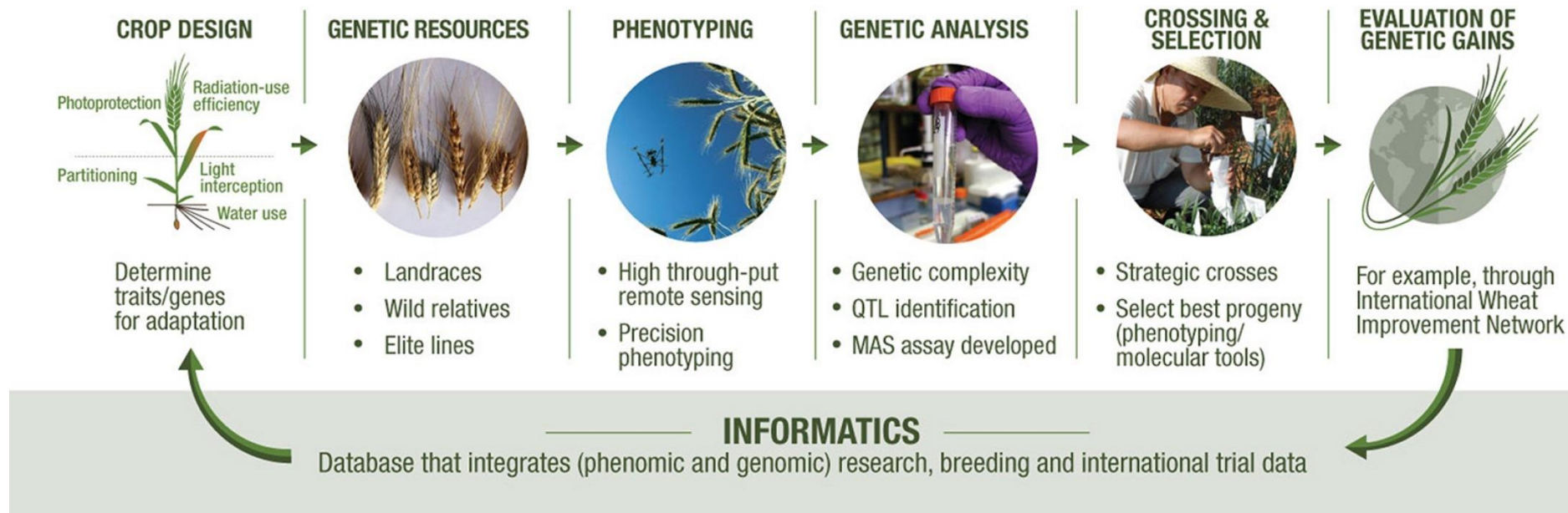


Genomic

Physiological pre-breeding

Objective: Improve abiotic stress adaptation and yield potential in a changing climate

PHYSIOLOGICAL BREEDING PIPELINE



Physiological breeding as strategy for genetic gain: use of conceptual models

YIELD POTENTIAL



$$\text{YIELD} = \text{LI} \times \text{RUE} \times \text{HI}$$

HEAT



$$\text{YIELD} = \text{LI} \times \text{RUE} \times \text{HI}$$

DROUGHT



$$\text{YIELD} = \text{WU} \times \text{WUE} \times \text{HI}$$



Conceptual Model of Heat-Adaptive Traits

$$\text{YIELD} = \text{LI} \times \text{RUE} \times \text{HI}$$

Photo-Protection (RUE)

- Leaf morphology (display, wax)
- Down regulation
- Pigment composition
 - Chl a:b
 - Carotenoids
- Antioxidants

Partitioning (HI)

- Spike fertility (meiosis, pollen, etc)
- Stress signaling (e.g. ethylene) regulating
 - senescence rate
 - floret abortion
- Grain filling (starch synthase)
- Stem carbohydrate storage & remobilization

Efficient metabolism (RUE)

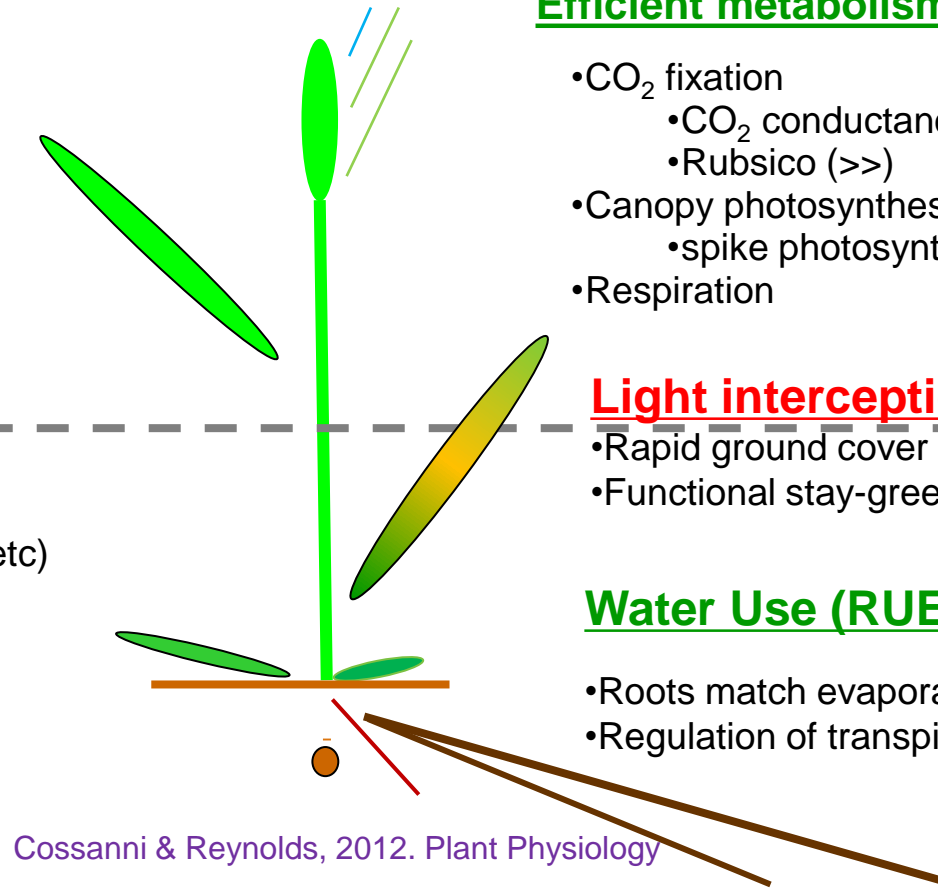
- CO₂ fixation
 - CO₂ conductance
 - Rubisco (>>)
- Canopy photosynthesis
 - spike photosynthesis
- Respiration

Light interception (LI)

- Rapid ground cover
- Functional stay-green

Water Use (RUE)

- Roots match evaporative demand
- Regulation of transpiration (VPD; ABA)



Cossanni & Reynolds, 2012. Plant Physiology

Traits considered in strategic crosses (to date):

$$\text{YIELD} = \text{LI} \times \text{RUE} \times \text{HI}$$

SOURCE (PRE-grainfilling):

- **Light interception (LI)**
 - ceptometer
 - NDVI
- **Source:sink balance**
- **Growth rate (RUE)**
 - Growth analysis/rate
 - canopy temp (CT)
 - leaf conductance (cond)
 - NDVI

SINK (PRE-grainfilling):

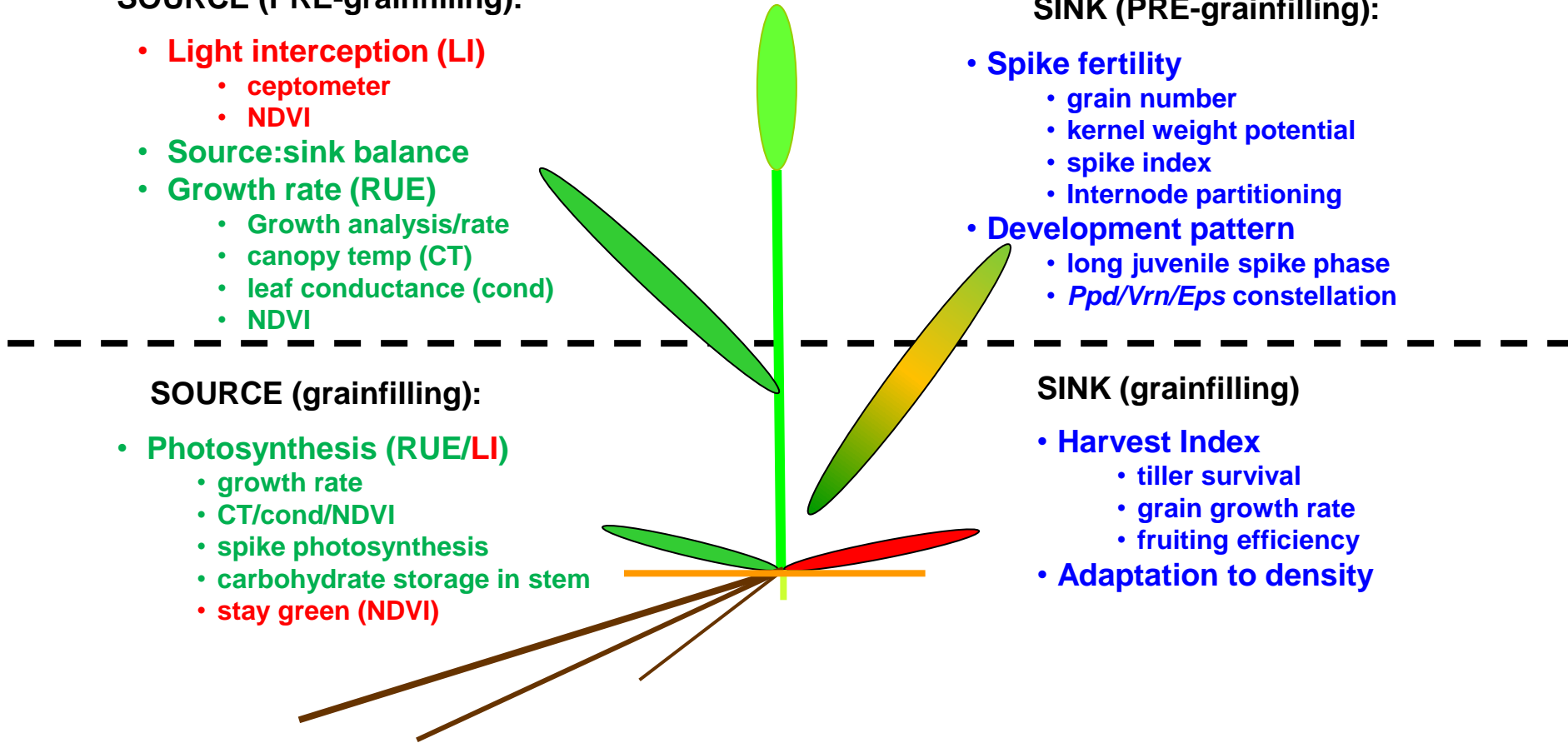
- **Spike fertility**
 - grain number
 - kernel weight potential
 - spike index
 - Internode partitioning
- **Development pattern**
 - long juvenile spike phase
 - *Ppd/Vrn/Eps* constellation

SOURCE (grainfilling):

- **Photosynthesis (RUE/LI)**
 - growth rate
 - CT/cond/NDVI
 - spike photosynthesis
 - carbohydrate storage in stem
 - stay green (NDVI)

SINK (grainfilling)

- **Harvest Index**
 - tiller survival
 - grain growth rate
 - fruiting efficiency
- **Adaptation to density**



Breeding friendly phenotyping

Breeding friendly phenotyping in GWP



1. Low resolution stereoscopic spectral radiometer (eyes) + supercomputer (brain).

Trait class / Approach:

Handy-visual

Application / Traits:

Phenology, canopy architecture, disease, pests



2. Greenseeker for NDVI.

3. IR thermometer for canopy temperature.

Trait class / Approach:

Handy-physiological

- NDVI/SPAD
- IR thermometer

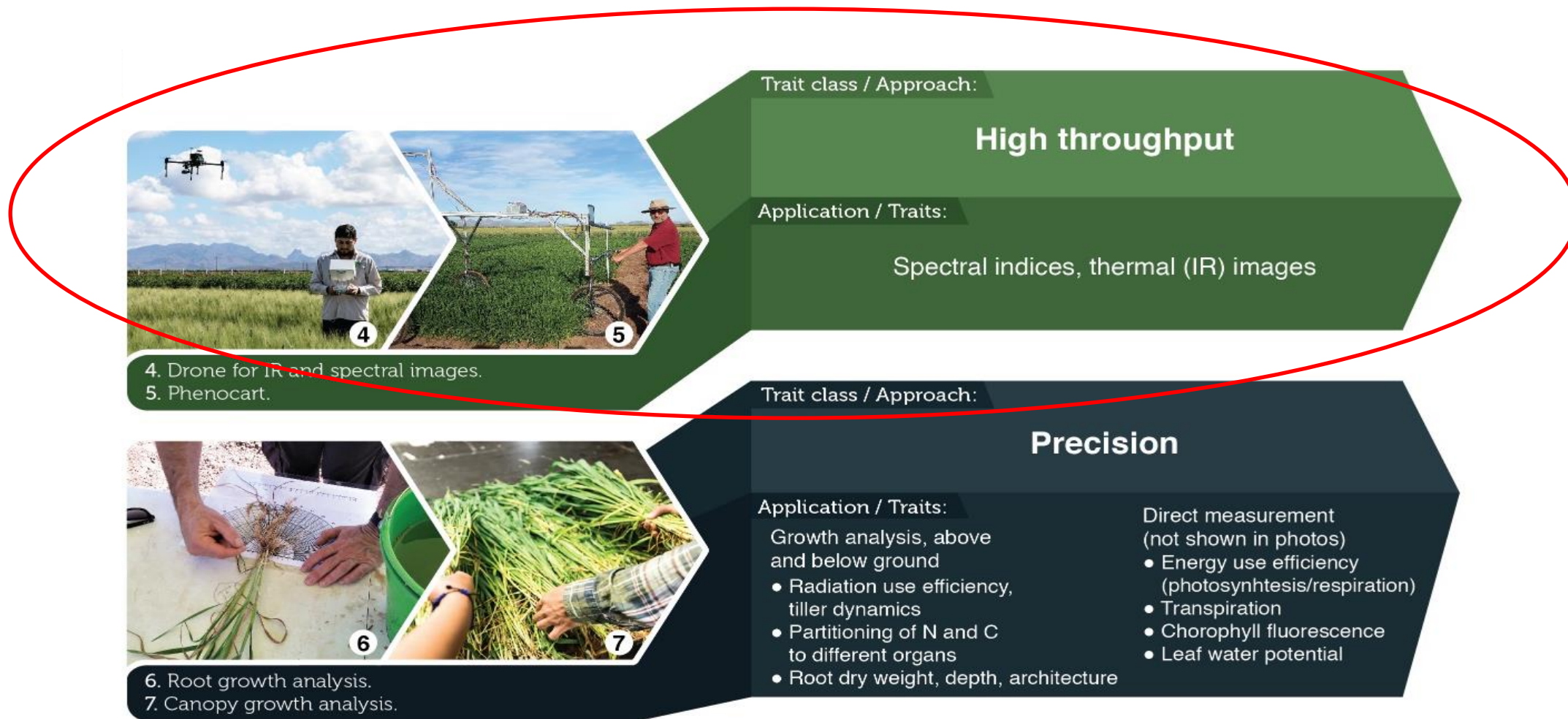
Application / Traits:

Ground cover, green area, biomass, leaf greenness

Canopy temp: fitness, root depth/capacity

Reynolds et al. 2020

Breeding friendly phenotyping in GWP



Reynolds et al. 2020

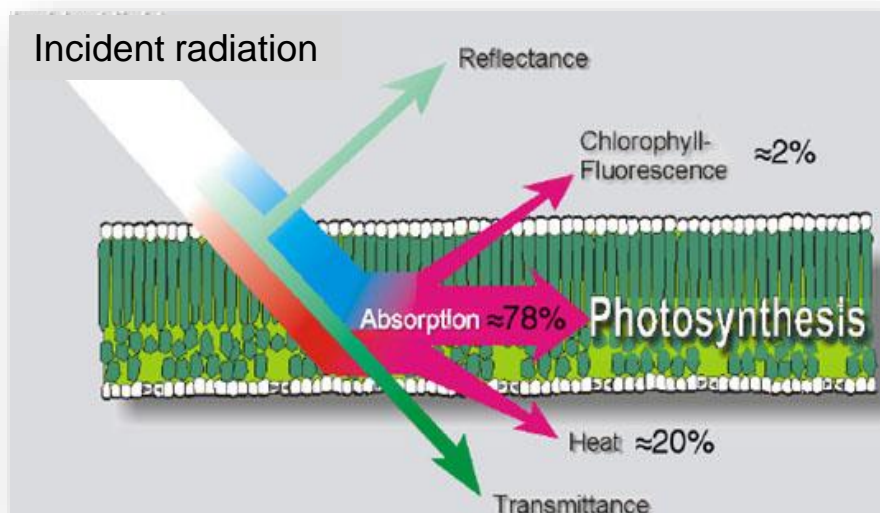
RS and sensors for HTP under field conditions

Advantages:

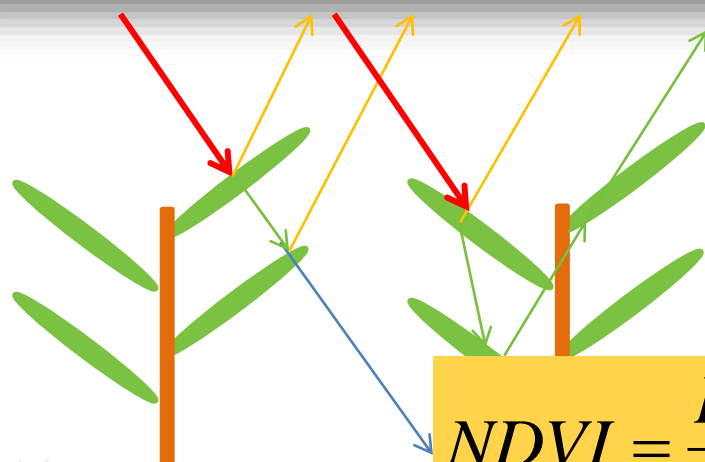
- Non-invasive
- Multiple spatio-temporal dimensions
- Fast
- Systematic data collection (reduced errors and allows automation)
- It can potentially be cost effective



1) Spectroscopy for plant phenotyping

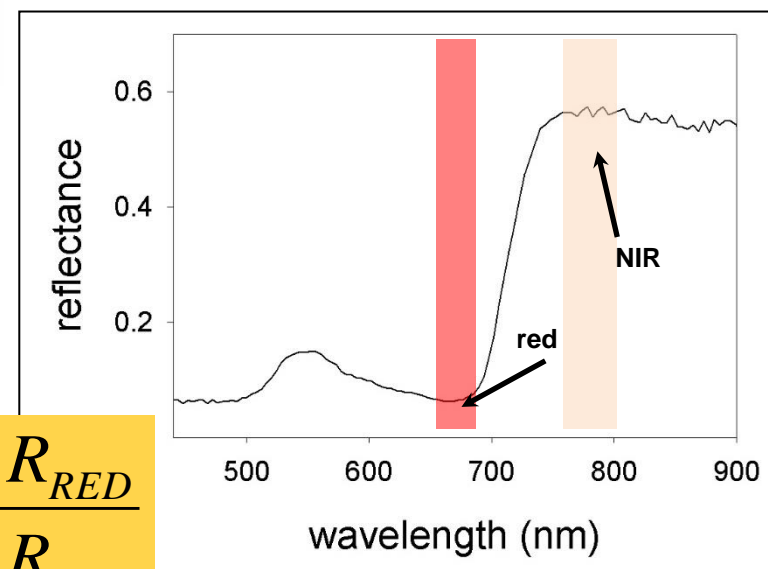


Spectral patterns of absorption, transmission and reflection of photons are primarily determined by plant pigments, constituents and structure

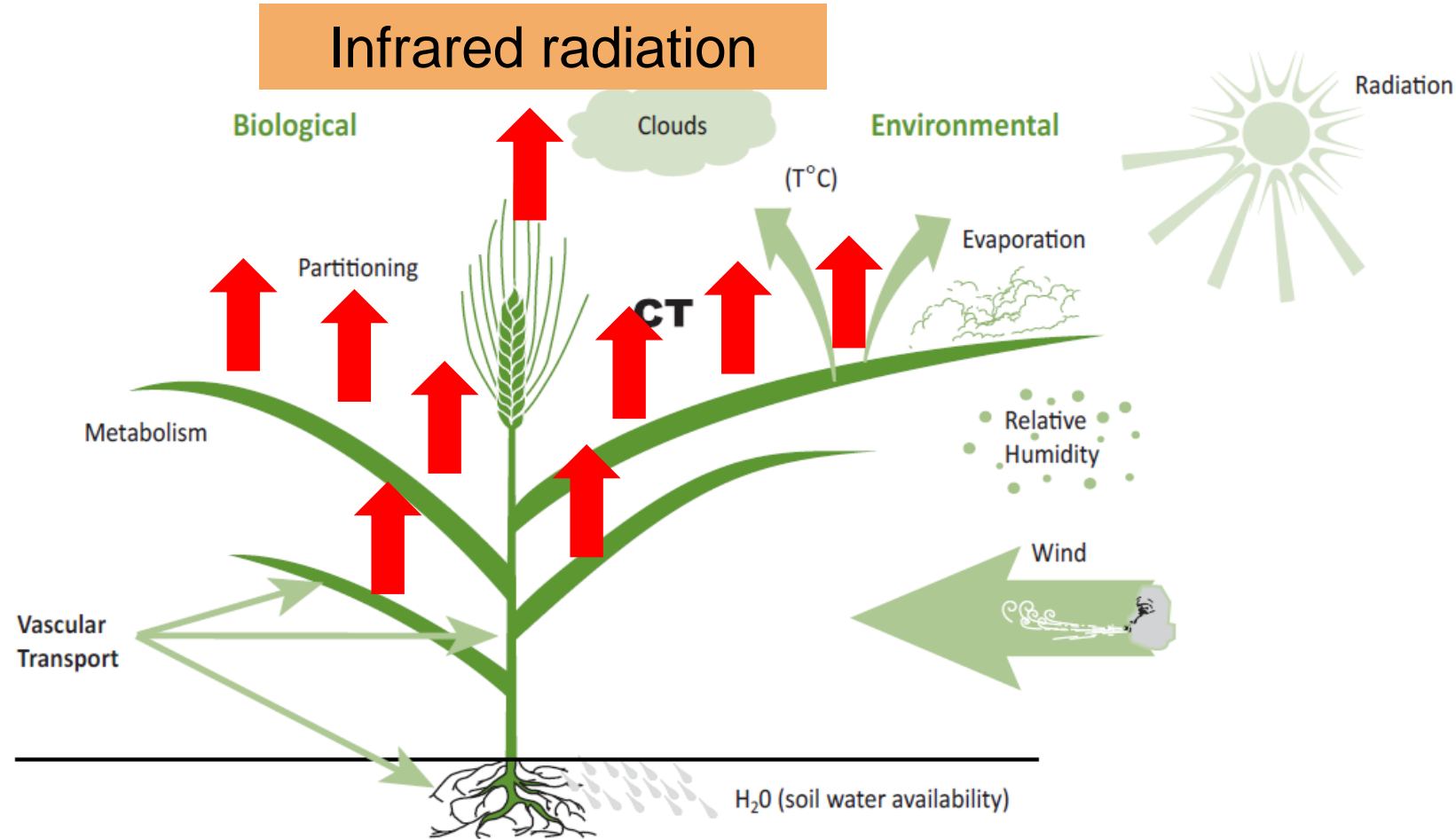


Canopy Radiative Trans

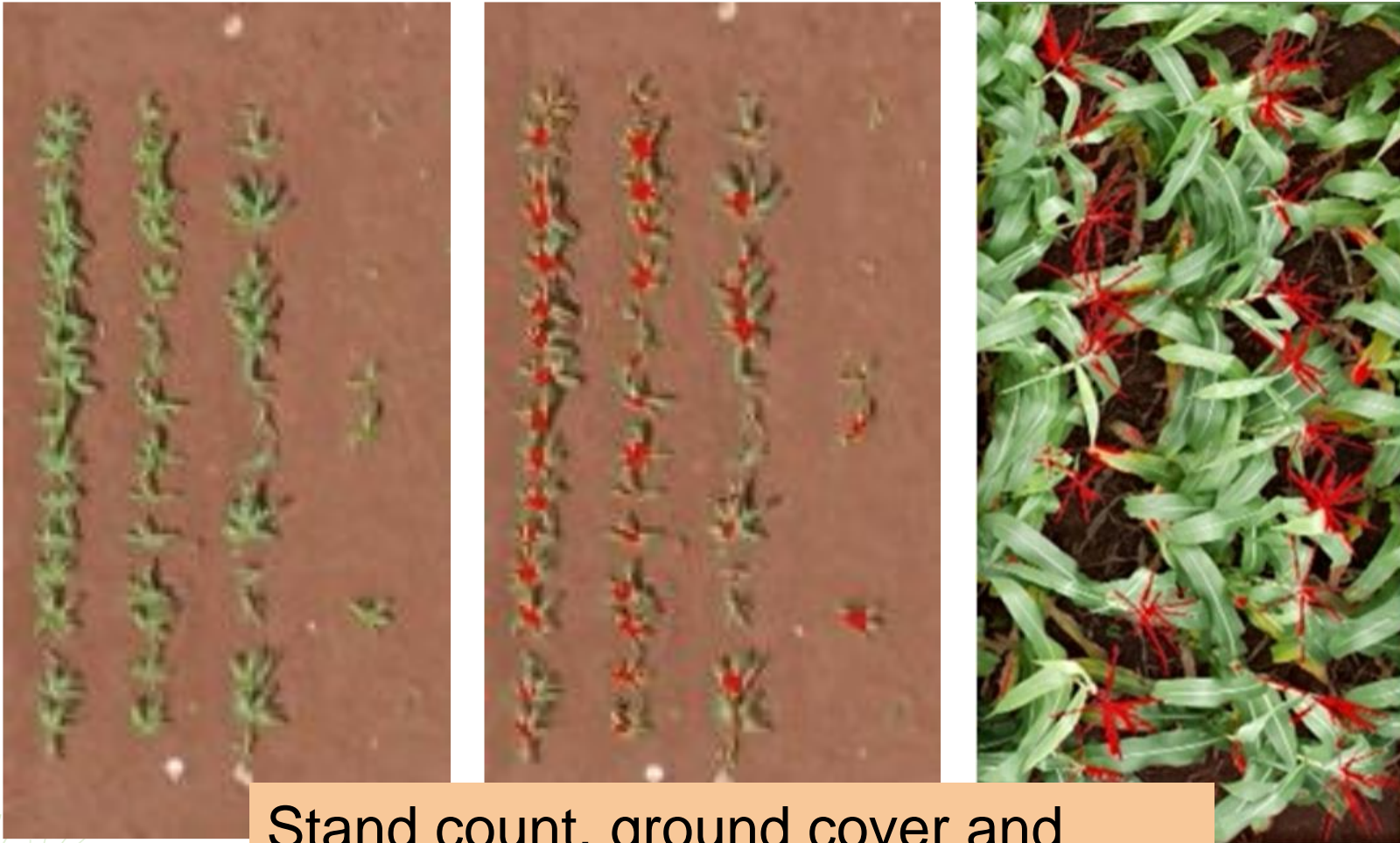
$$NDVI = \frac{R_{NIR} - R_{RED}}{R_{NIR} + R_{RED}}$$



2) Thermography: plant temperature as an indicator of physiological status



3) Methods for object identification and classification + photogrammetry



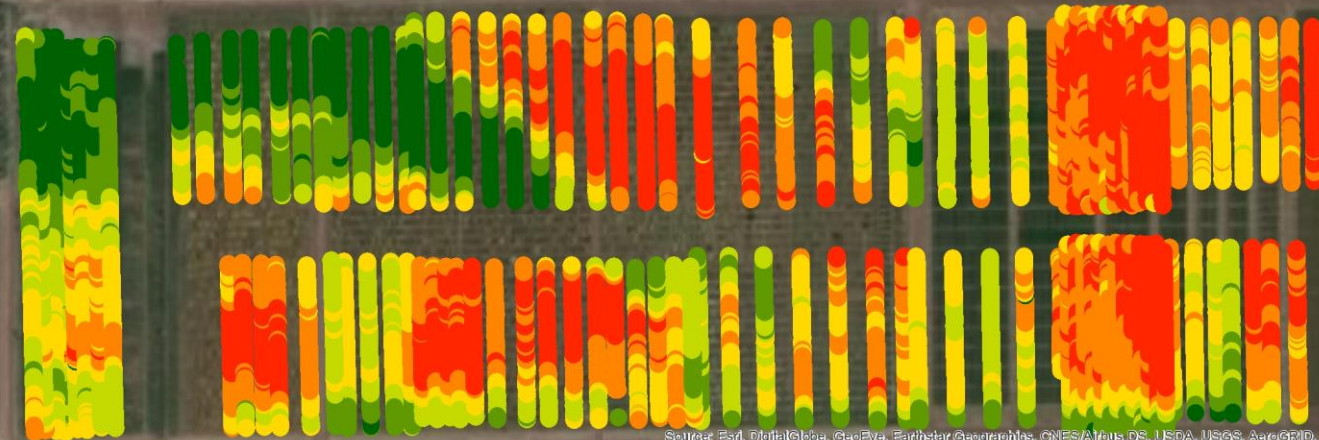
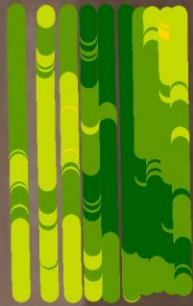
Stand count, ground cover and phenology using RGB imaging

4) Envirotyping

- Soil characterization
- Weather characterization
- Field management

Electrical Conductivity

| | |
|---|-------------------------|
| ● | 36.7149514 - 67.871000 |
| ● | 67.871001 - 82.531000 |
| ● | 82.531001 - 94.455000 |
| ● | 94.455001 - 105.851000 |
| ● | 105.851001 - 116.945000 |
| ● | 116.945001 - 139.992000 |



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community



Platforms and sensors

Sensors and platforms: aerial phenotyping at GWP

Drones



Matrice 200
DJI, China

Max. payload: **1 kg**



Matrice 600
DJI, China

Max. payload: **6 kg**



eBee senseFly,
Switzerland

Max. payload: **0.15 kg**

NDVI and vegetation indices



RedEdge, MicaSense
Bands: 475, 560, 668,
840, 717 nm

Main products: **NDVI, SR**
GSD at 30m: 2.0 cm/pixel



2p multispec, Slanrange
Bands: 532, 570, 650,
850 nm

Main products: **PRI, NDVI**
GSD at 30m: 1.3 cm/pixel



ADC-Lite, Tetracam
Bands: 560, 660, 830 nm
Main product: **NDVI**
GSD at 30m: 1.1 cm/pixel



Mini-MCA 12 ch, Tetracam
Bands: 445, 550, 670, 680,
700, 710, 720, 760, 780,
800, 900, 970 nm

Main products: **NDVI, WI, SR, RARS**
GSD at 30m: 1.6 cm/pixel

Thermography



Zenmuse XT, DJI/FLIR
Range: 8-14 μ m
GSD at 30m:
4 cm/pixel



TAU 640, FLIR
Range: 8-14 μ m
GSD at 30m:
2 cm/pixel



Zenmuse X5, DJI
GSD at 30m:
0.8 cm/pixel



NEX 5, SONY
GSD at 30m:
0.9 cm/pixel

RGB y 3D

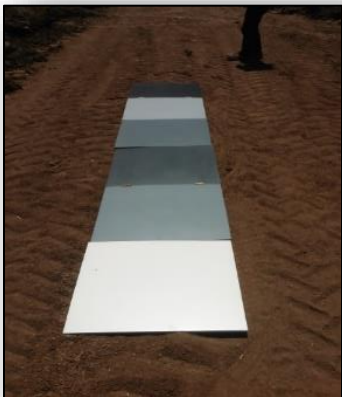
Hyperspectral



Micro-Hyperspec, Headwall Photonics
Range: 600 – 1700 nm
n. bands: 267
GSD at 30m: 1.8 cm/pixel

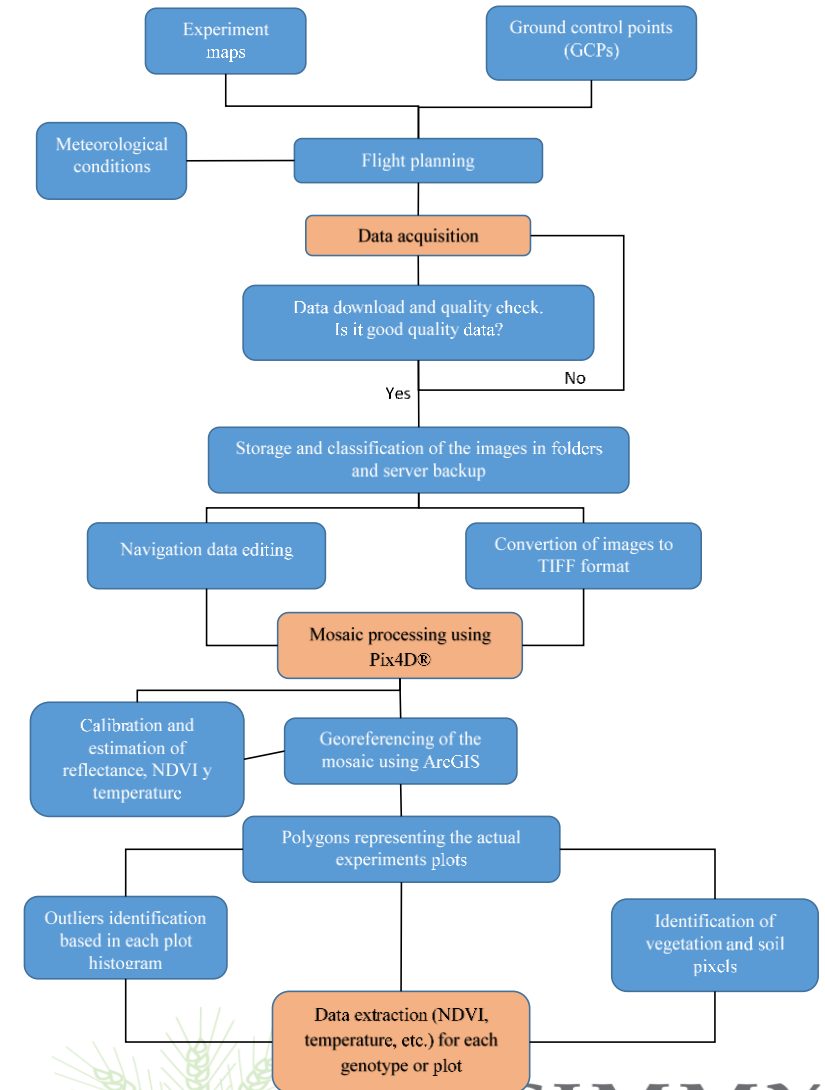
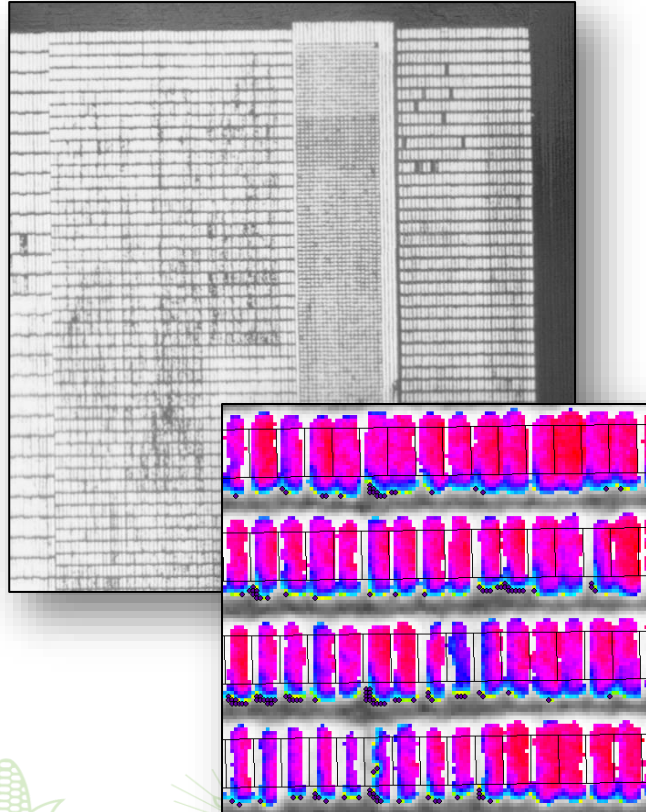
Towards the automation of a UAV-based HTP platform

Georeferencing our experiments



Images generated by J.M. Mendoza

Semi-automatic data extraction



Moving from UAV to manned aircraft

Multispectral and thermal cameras:

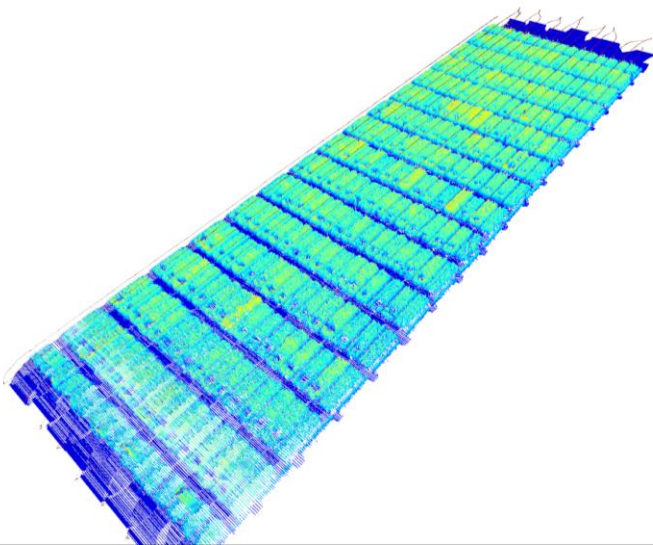
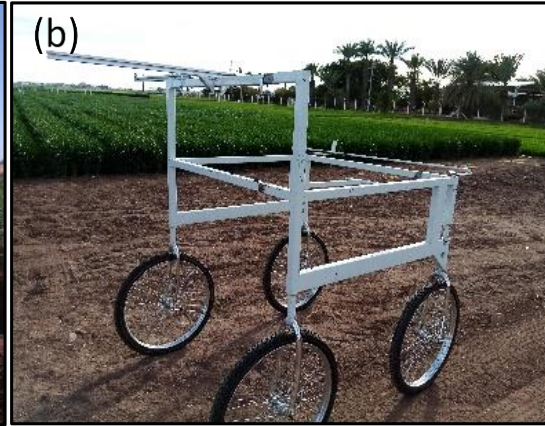
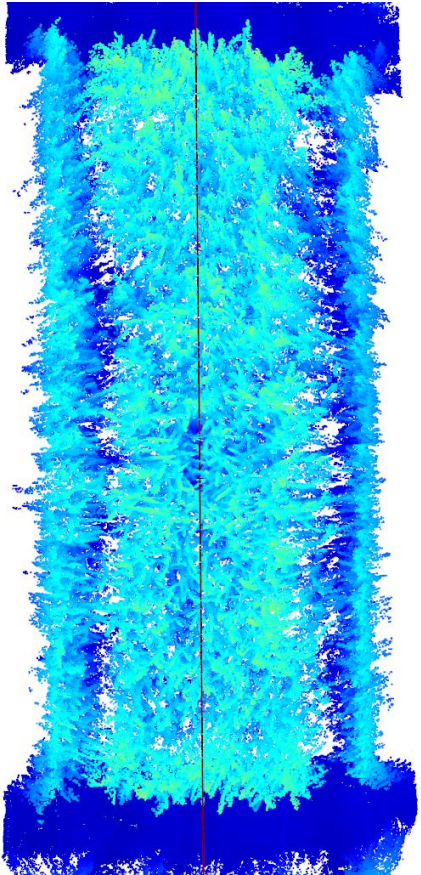
- NDVI
- MCARI
- Chlorophyll Index (green)
- Chlorophyll Index (Red-Edge)
- LAI (Prosail)
- CAB(Prosail)
- FAPAR (Prosail)
- QPAR
- Canopy Temperature
- Biomass index
- Plant height

(Collaboration with HIPHEN)

Flights every
7-10 days



New platforms for ground phenotyping



Development of a terrestrial vehicle for integrated measurements of different sensors:

- LIDAR: structural traits such as plant height and biomass.
- Thermal sensors
- Multispectral cameras and hyperspectral sensors
- RGB digital cameras
- NDVI and PRI sensors

New platforms for ground phenotyping



Dataset for the:
**The Global Wheat
Head Detection
Challenge**



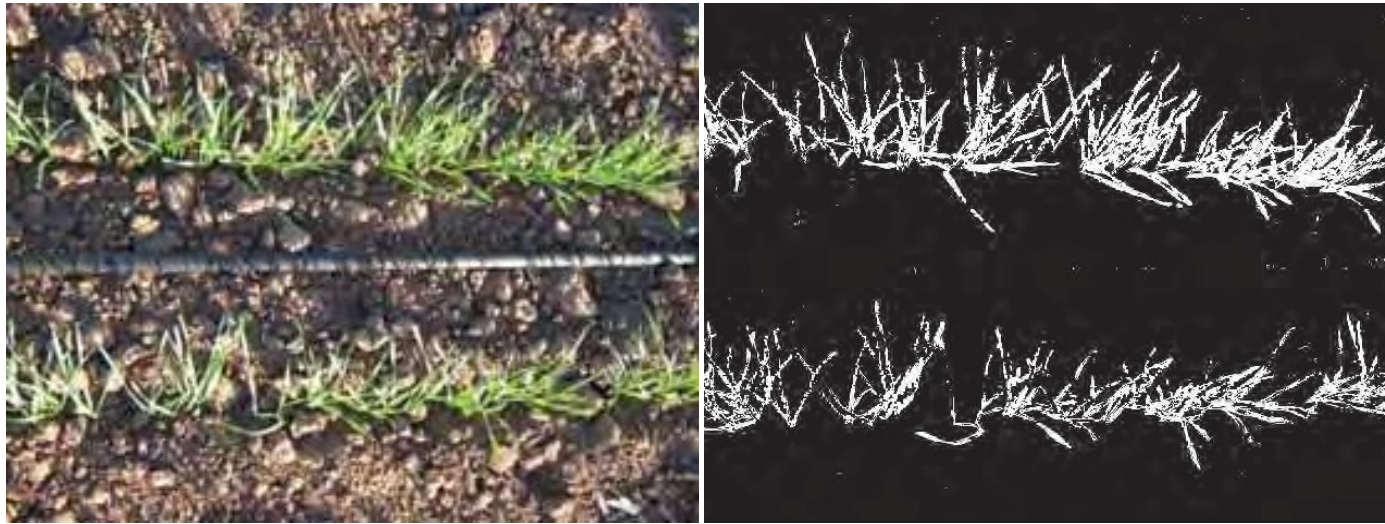
Collaboration with private sector
for developing a phenotyping
rover based on Deep Learning



Evaluating some key physiological traits

Digital RGB images

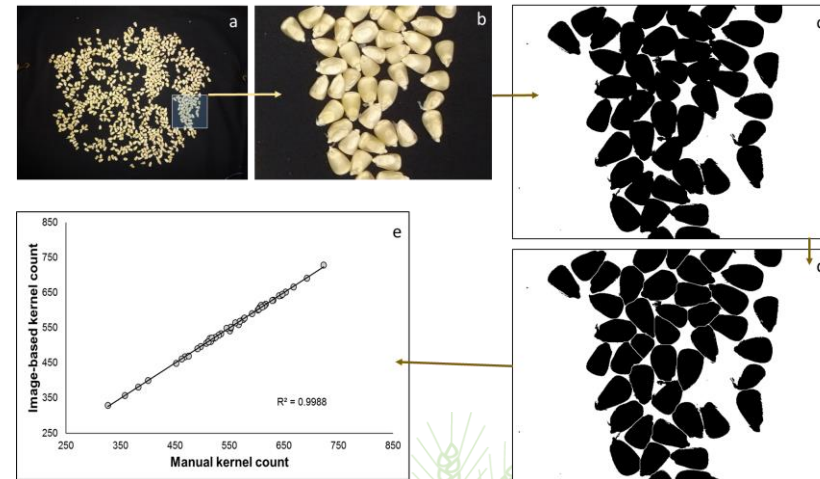
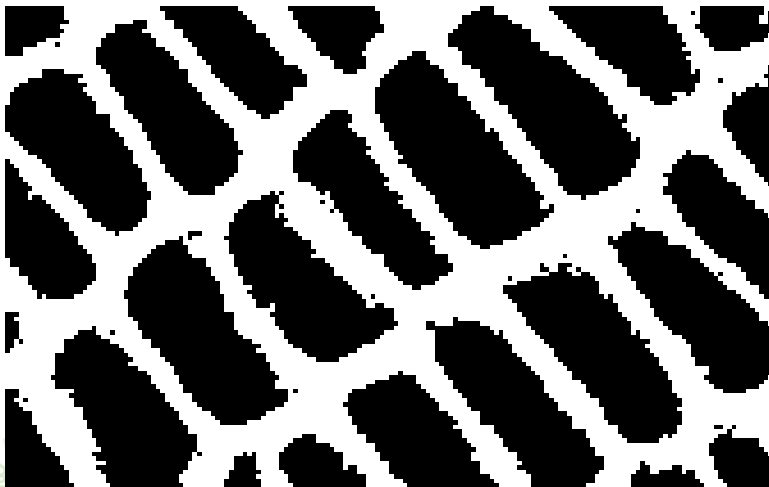
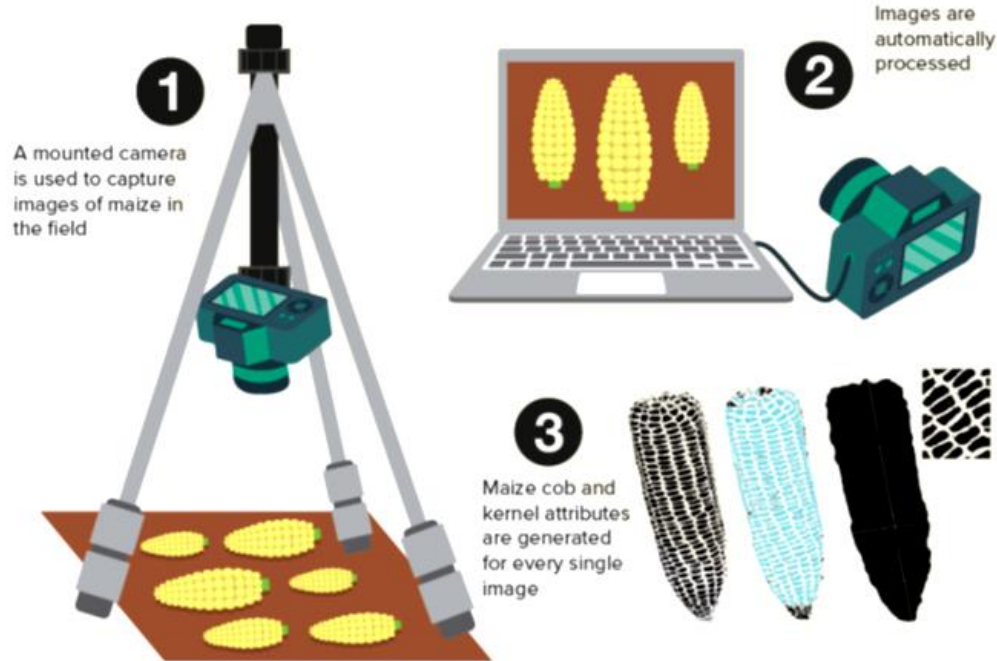
Using RGB imaging for spatial distribution and structural properties of the vegetation



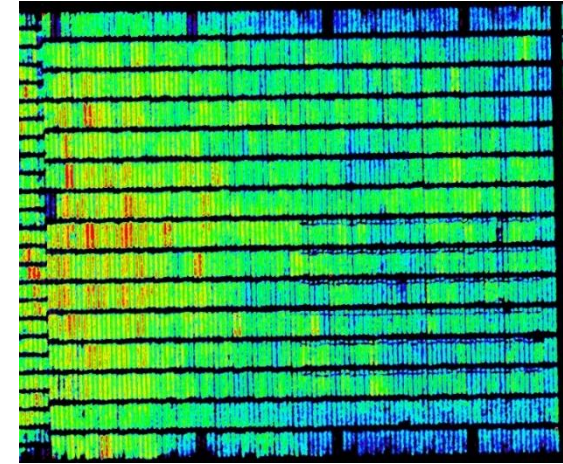
Calculating the vegetation ground cover using.



Maize Ear Phenotyping

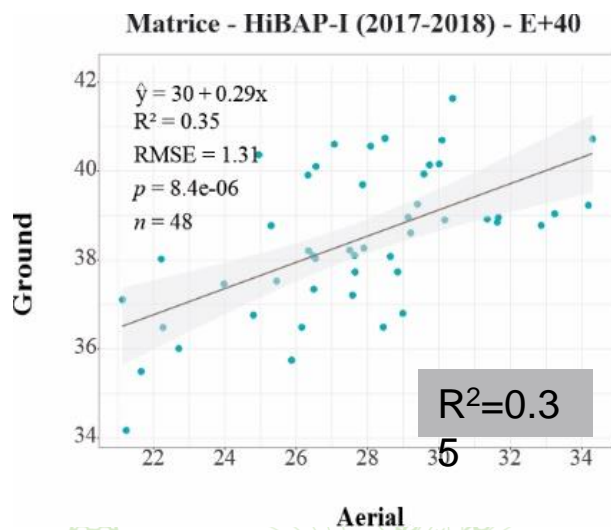


RGB photogrammetry for reconstruction of 3D model of canopy surface

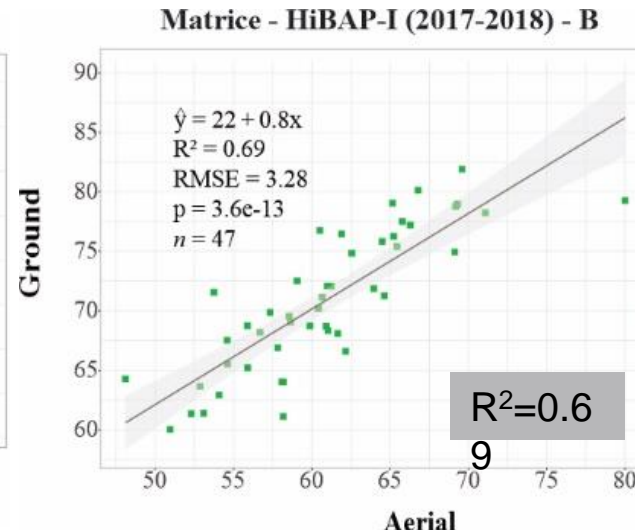


- Good estimations of plant height.
- Prospects for estimation of biomass using plot volume as proxy

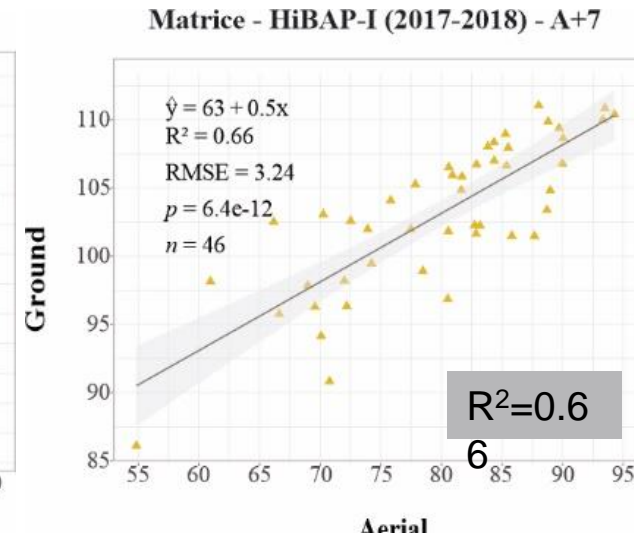
Volpato et al. (2021)



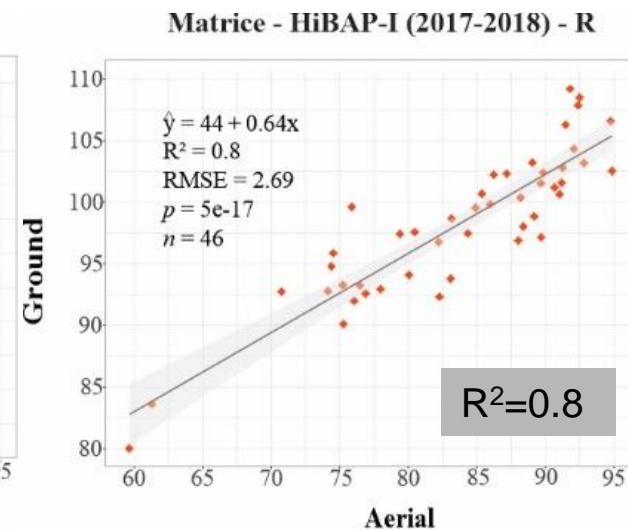
40 days after emergence



Booting



7 days after Anthesis



Physiological maturity

Estimation of RUE under yield potential

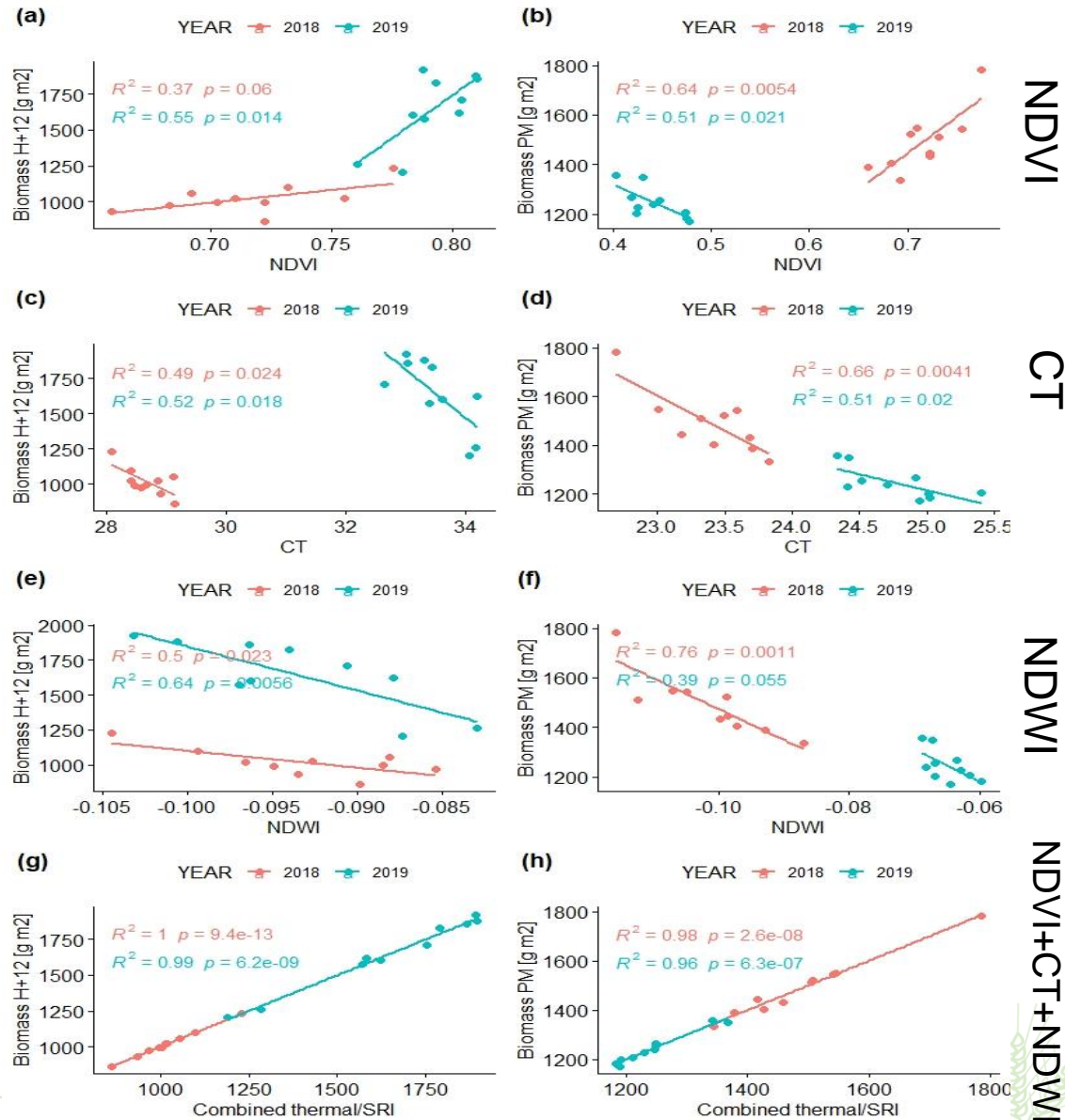
| Trait | Growth stage | RS variables used in the best models | R ² | RMSE |
|---------|-------------------|--------------------------------------|----------------|-------|
| IPAR | E+40 | NDVI, GNDVI, MCARI | 0.95 | 3.21 |
| | A+7 | NDVI | 0.92 | 11.99 |
| | PM | NDVI | 0.92 | 20.93 |
| Biomass | E+40 | CRI, CUR, NDVI, TCARI | 0.63 | 16.06 |
| | A+7 | NDWI, CT, CT | 0.66 | 51.73 |
| | PM | CT, NDVI | 0.61 | 74.17 |
| RUE | pre Grain Filling | NDVI, NDWI | 0.42 | 0.17 |
| | grain filling | SPAD, CT, EVI, RGR | 0.79 | 0.15 |
| | Total | SPAD, CRI, CT, NDVI | 0.94 | 0.04 |



- IPAR: chlorophyll-related indices (e.g. NDVI)
- Biomass:
 - E+40: **Pigment-related indices** (TCARI and CRI)
 - A+7: **NDVI + normalized water index** (NDWI) + **canopy temperature** (CT)
- RUE:
 - Vegetative stage: **NDVI + NDWI**.
 - Grain filling: **CT + chlorophyll-related indices**

$$RUE = \frac{BM_{accumulated}}{IPAR_{accumulated}}$$

Estimation of shoot biomass



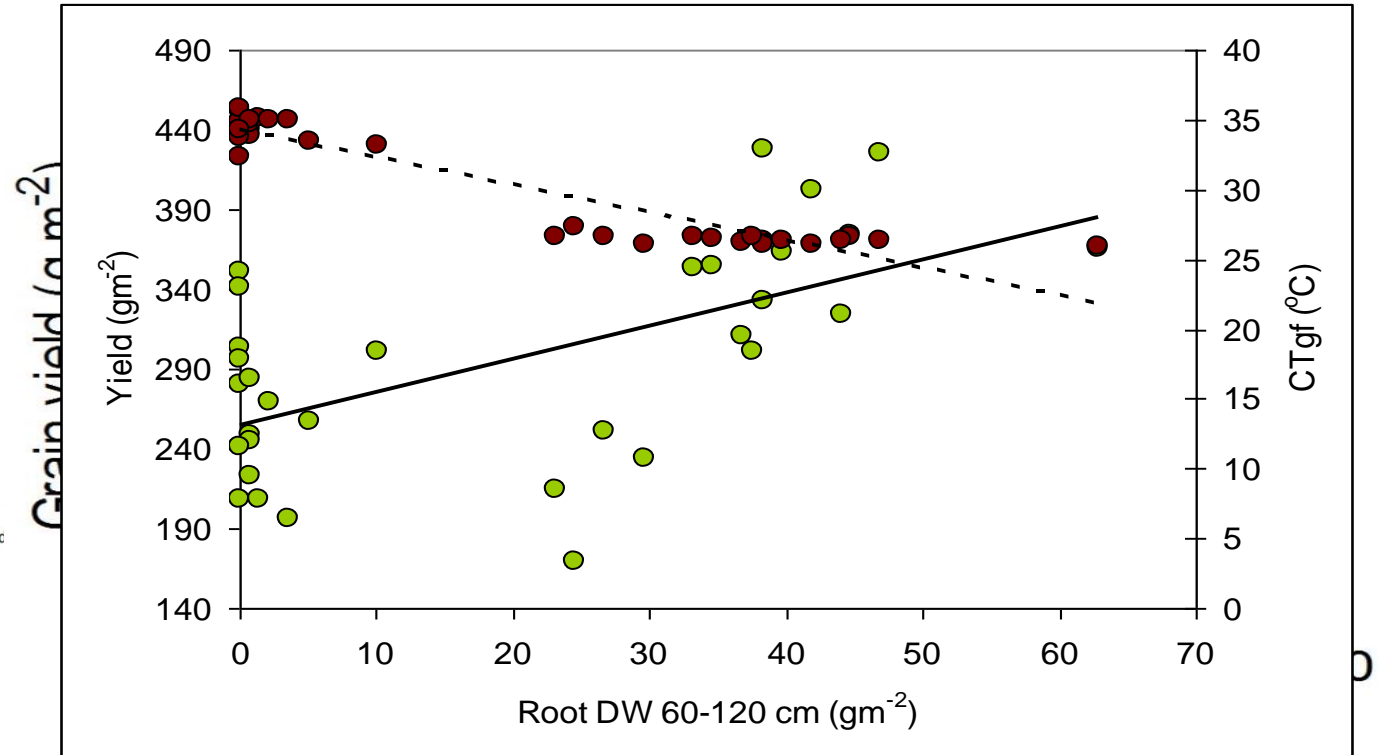
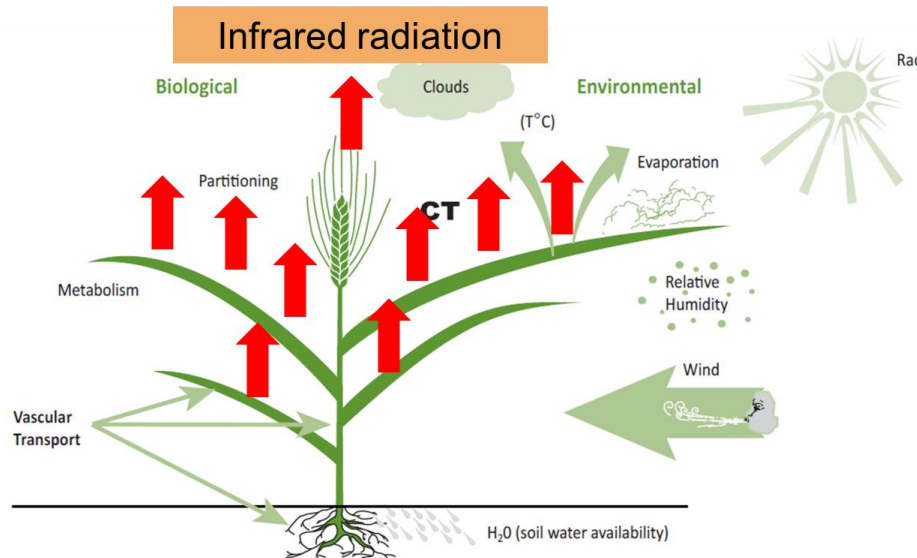
- Canopy temperature (CT), NDVI and water indices (NDWI) tested as estimators of biomass measured 12 days after heading (BM_H+12) and at physiological maturity (BM_PM)
- **NDVI** showed the lowest prediction capacity across dates.
- **CT** showed high correlation with both BM_H+12 and BM_PM, particularly in early grain filling
- **NDWI** also showed a robust and high correlation with biomass when measured during grain filling.
- The **multiple linear model** combining the three RS variables measured during grain filling resulted in the highest correlations with BM_H+12 and BM_PM

Next step: to build and validate similar model for a larger population and across years: HIBAP

Canopy temperature: a possibility for phenotyping root capacity

Canopy temperature:

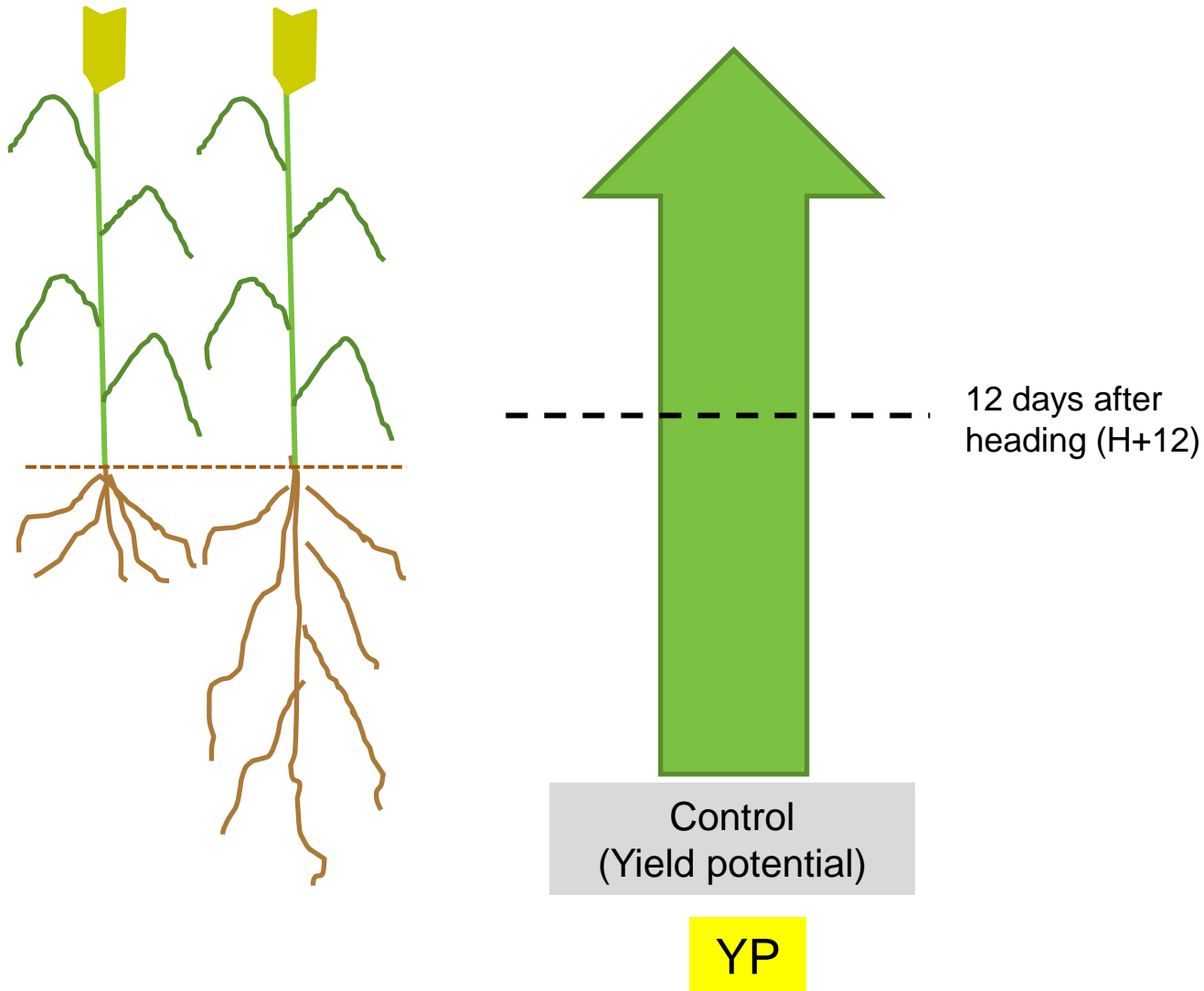
Related to the interaction of few environmental factors and plant physiology.



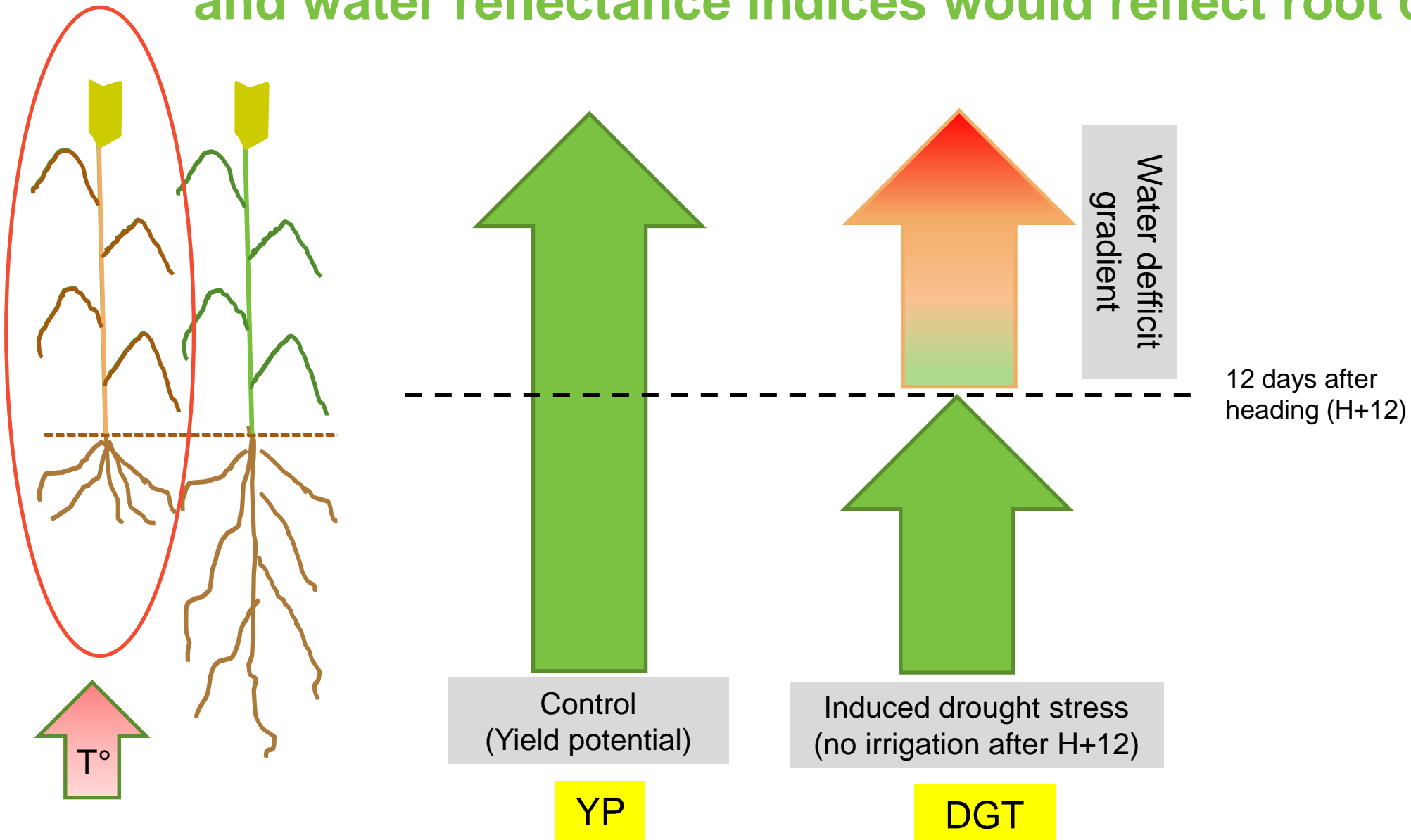
Lopes MS and Reynolds MP, 2010.

Pinto & Reynolds, 2015.

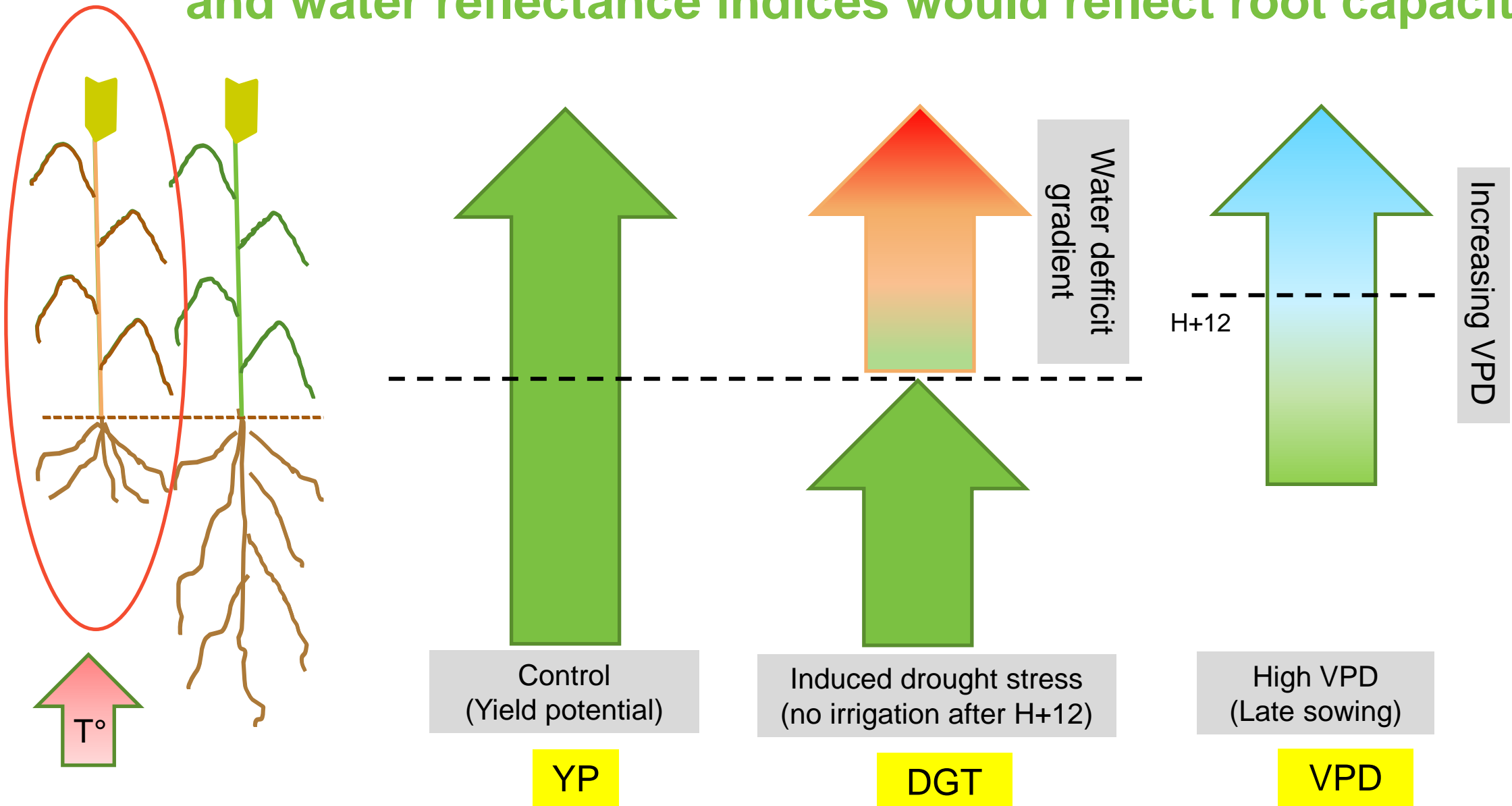
Field experiment: imposed stress where canopy temperature and water reflectance indices would reflect root capacity



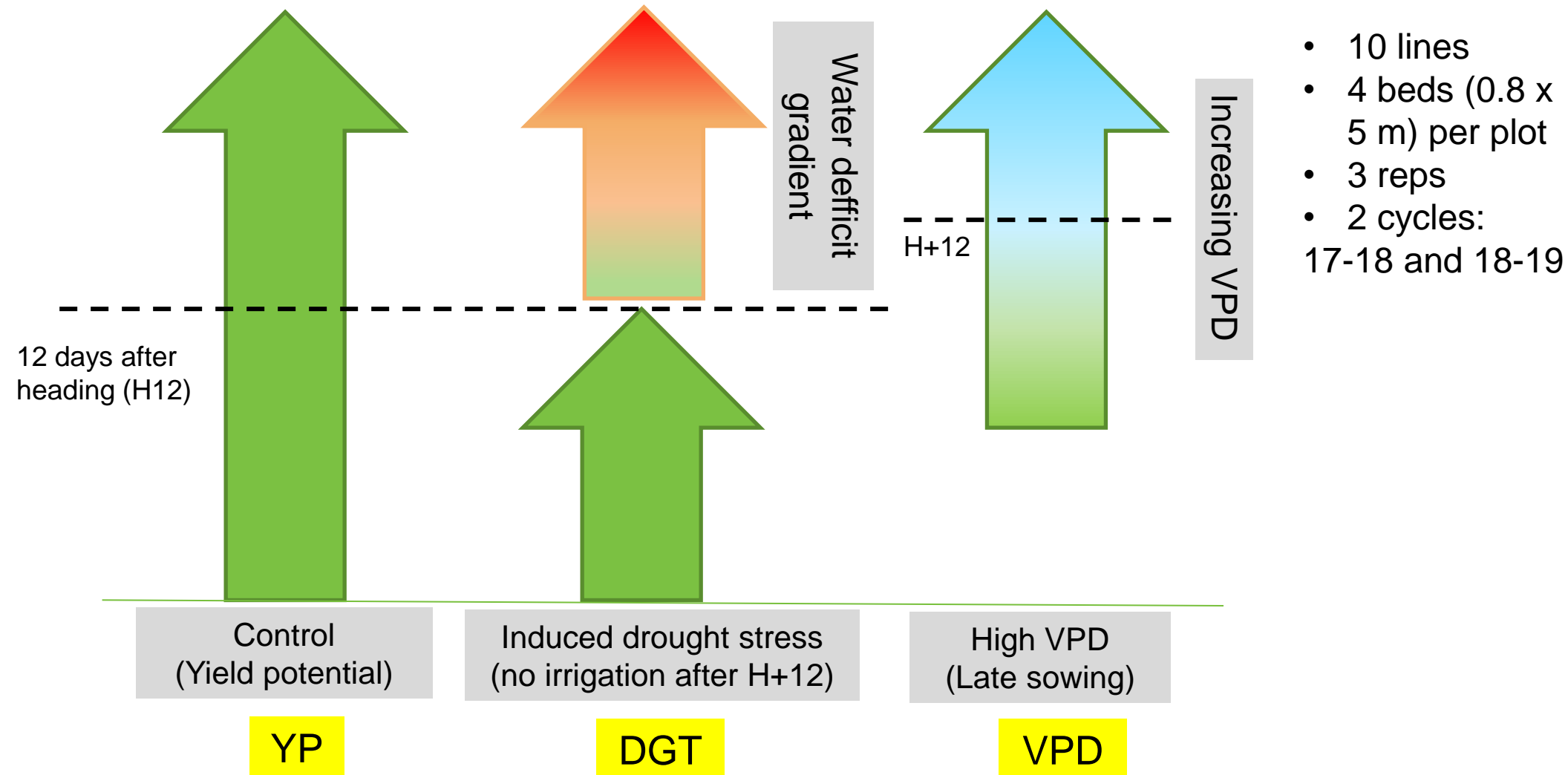
Field experiment: imposed stress where canopy temperature and water reflectance indices would reflect root capacity



Field experiment: imposed stress where canopy temperature and water reflectance indices would reflect root capacity



Field experiment: imposed stress where canopy temperature and water reflectance indices would reflect root capacity



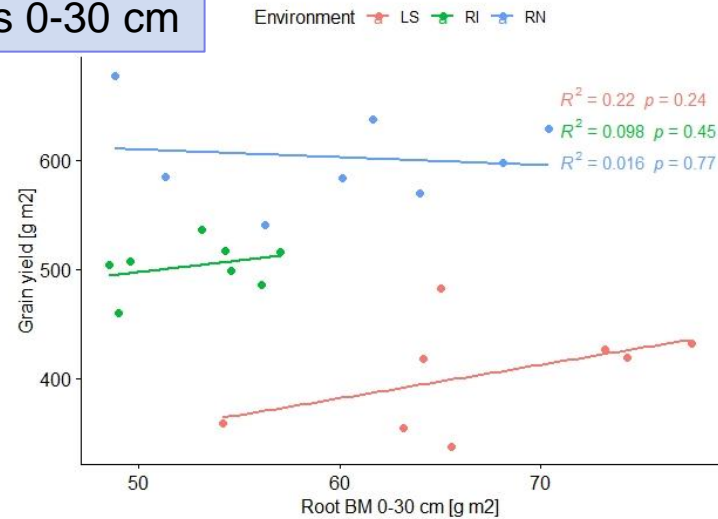
- 10 lines
- 4 beds (0.8 x 5 m) per plot
- 3 reps
- 2 cycles: 17-18 and 18-19



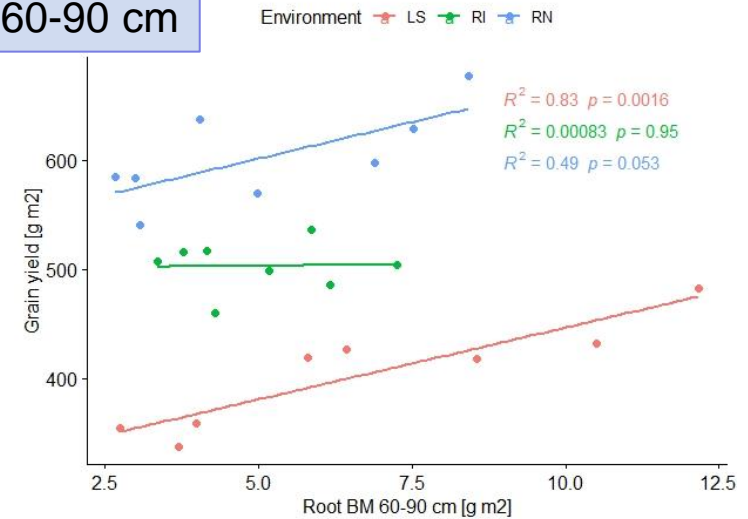
Root traits vs grain yield (2018)

Root biomass vs yield

Roots 0-30 cm



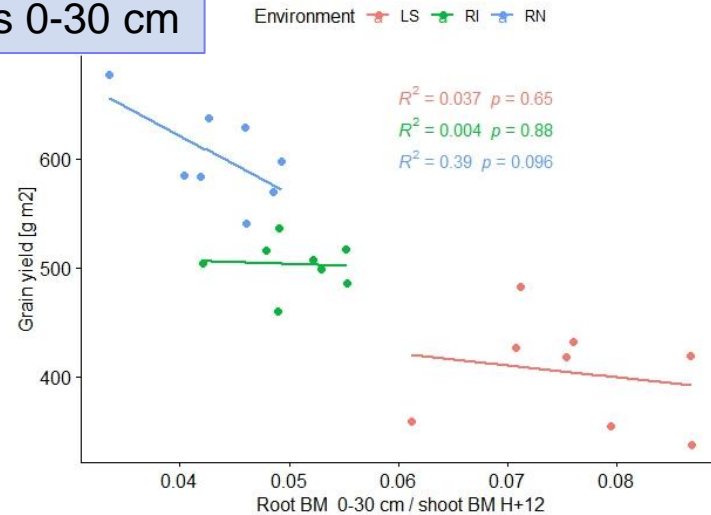
Roots 60-90 cm



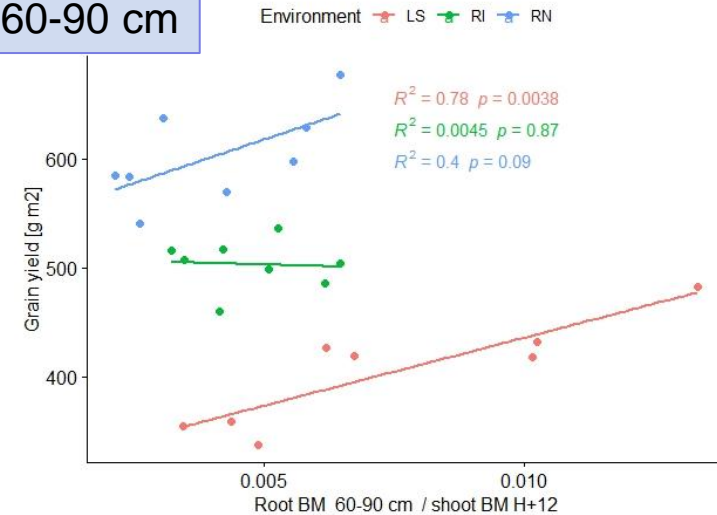
YP
DGT
VPD

Root : shoot vs yield

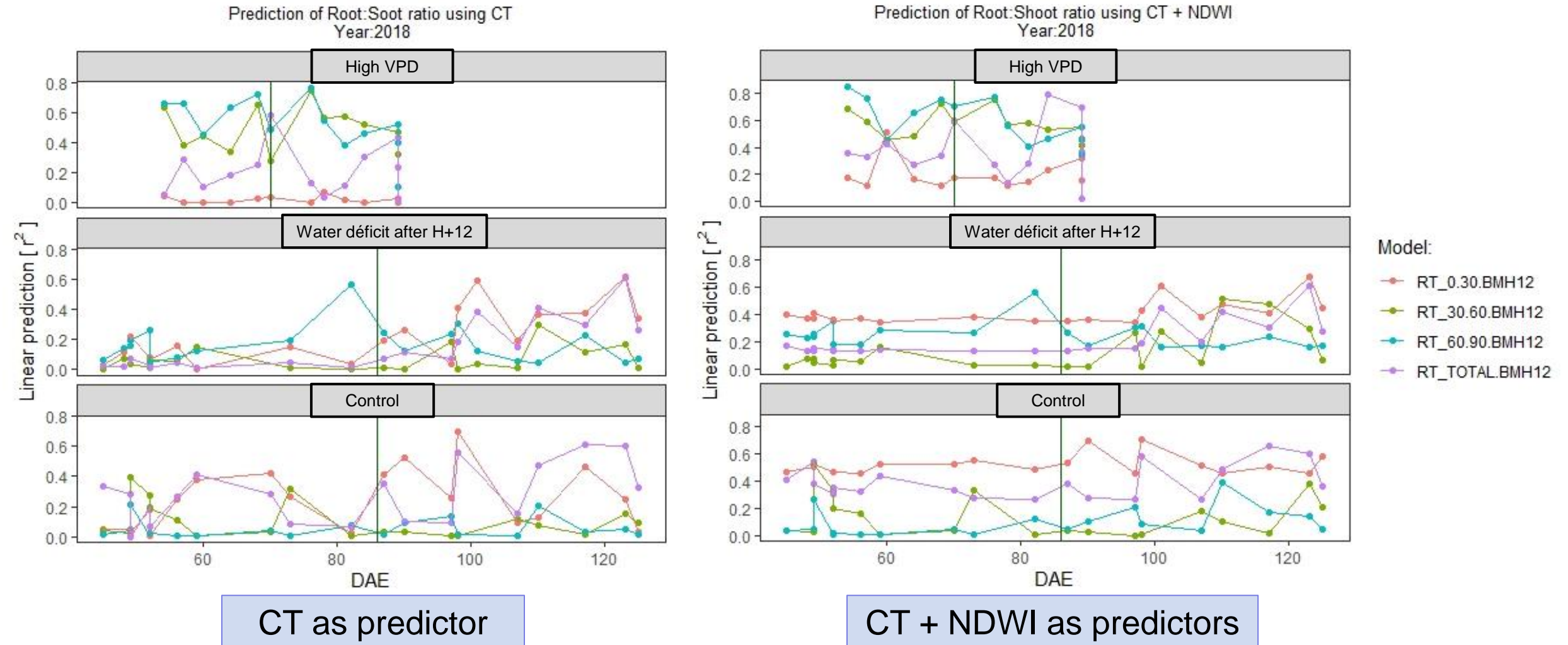
Roots 0-30 cm



Roots 60-90 cm

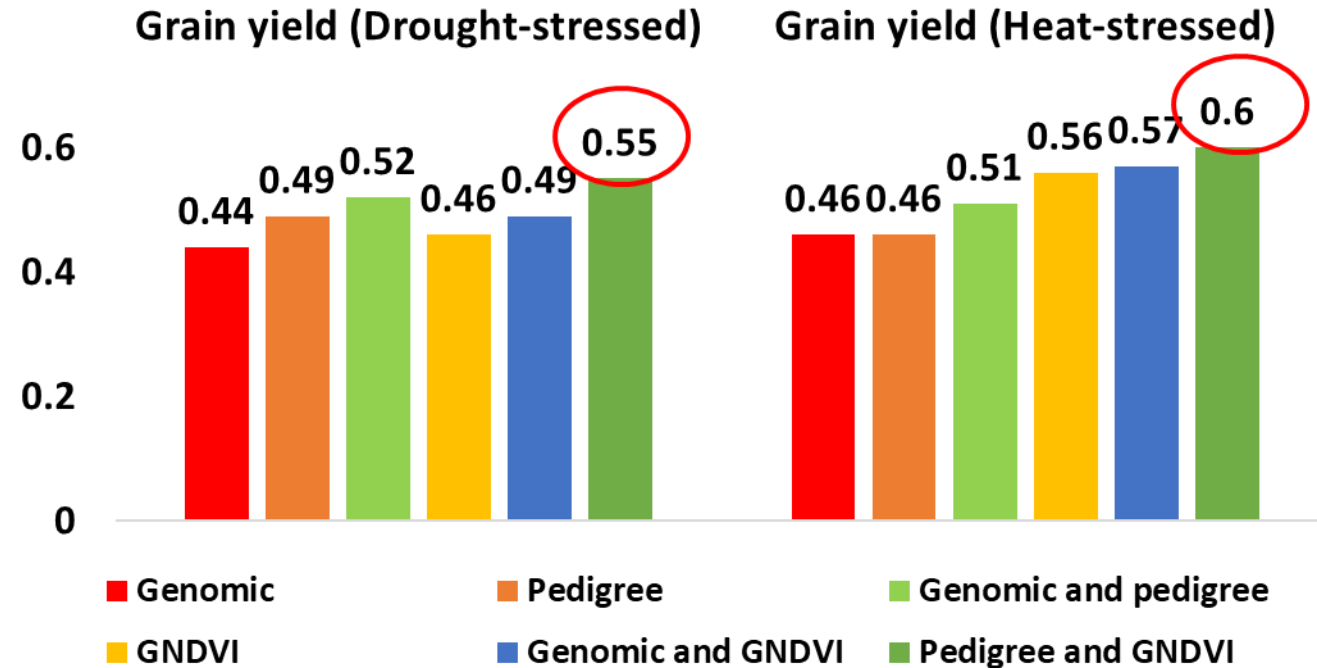


Canopy temperature and water reflectance indices as indicators of root:shoot ratio



HTP for improving genomic selection models

- Measuring NDVI and CT on YT and EYT
- Measurements on 3 cycles
- DGGW and Feed the Future Innovation Lab for Applied Wheat Genomics projects



NDVI for complementing genomic and pedigree selection models.

What is next...

Ongoing developments: challenges and “whish list”

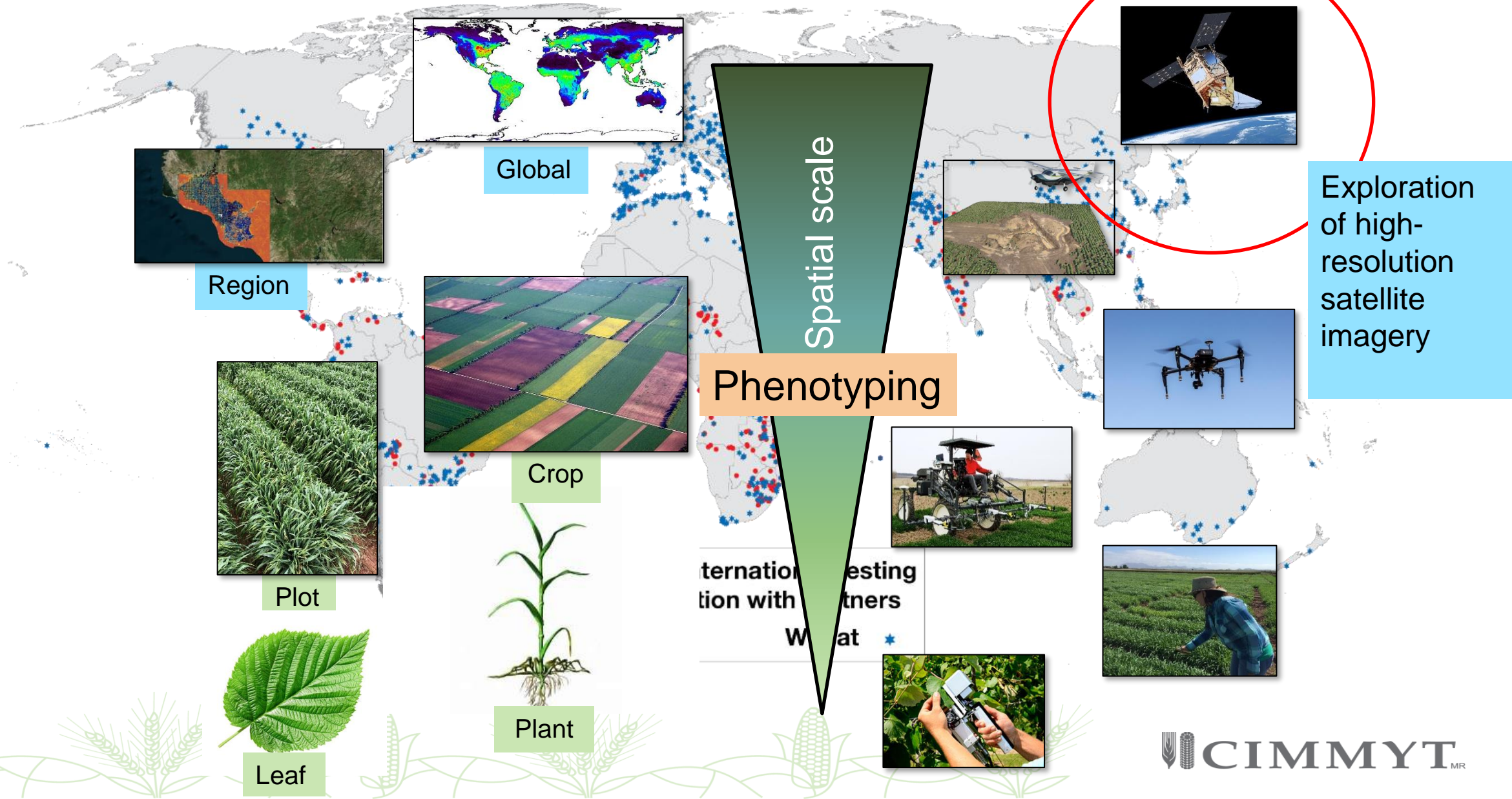
- Automatization and up-scaling of routine aerial of multispectral and thermal images (Collaboration with HIPHEN)
- Ground-based imaging systems for Deep-learning
- Improving environment characterization

Calibration/validation
Automatic image processing
Data quality check
Data management

Parameters/indices for indirect estimations of:

Biomass (water index, 3D point cloud)
Structural traits (3D reconstruction, radiative transfer models)
Water use efficiency
Radiation Use Efficiency (hyperspectral, fluorescence)
Phenology (heading detection)
Spike density
Disease screening and early detection

Challenges for MET in wheat: characterizing the GxExM component



Acknowledgments CIMMYT

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Matthew Reynolds, lead



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Hans Braun

Ravi Singh

Suchismita Mondal

Juliana Philomin

Gilberto Thompson

Rodrigo Rascon

SIP:

Francelino Rodrigues

Lorena Gonzalez

Donors & collaborators



BILL & MELINDA
GATES *foundation*



United States Department of Agriculture
National Institute of Food and Agriculture



KANSAS STATE
UNIVERSITY





**Thank you for
your interest!**