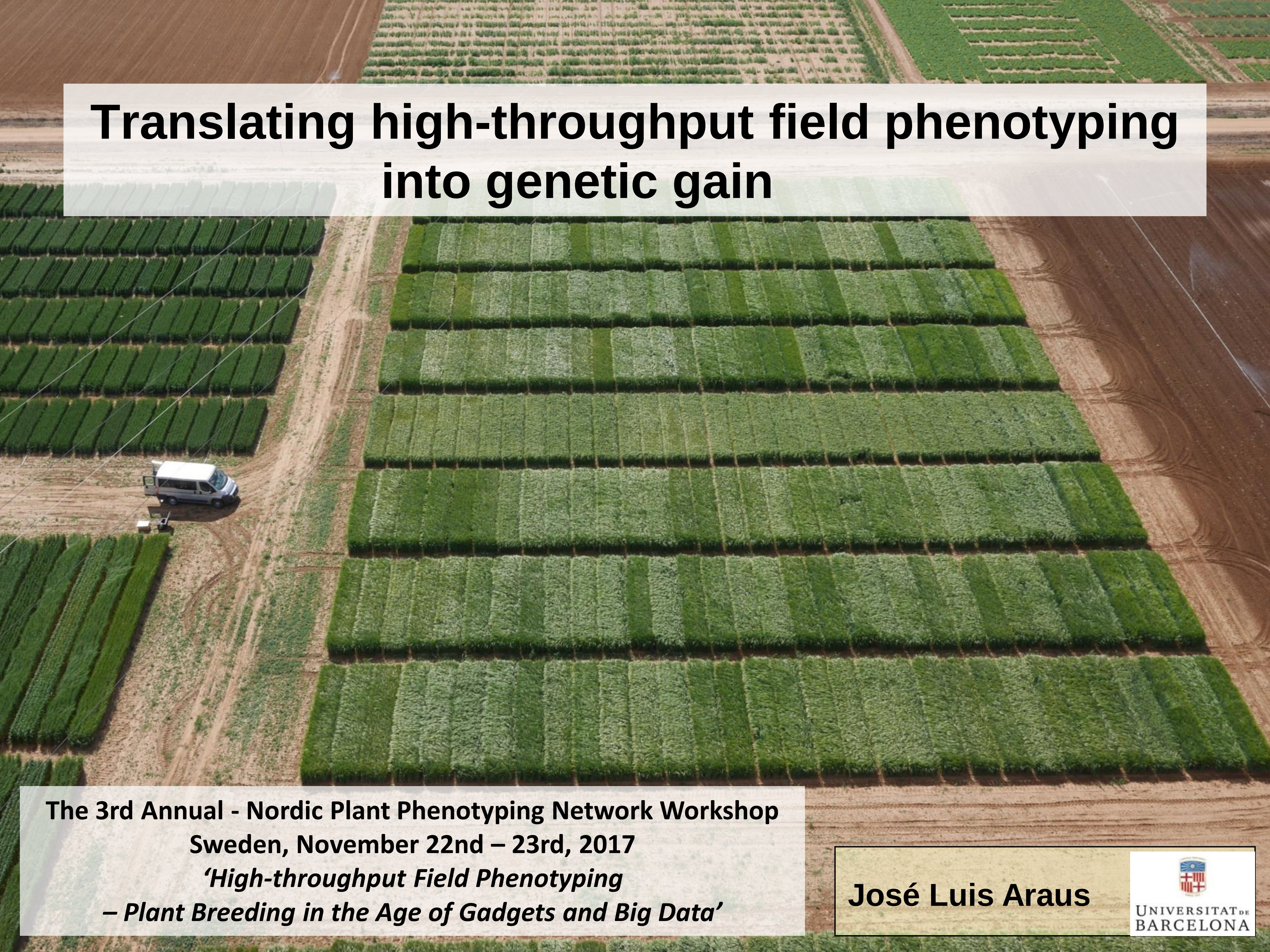

Translating high-throughput field phenotyping into genetic gain

The 3rd Annual - Nordic Plant Phenotyping Network Workshop
Sweden, November 22nd – 23rd, 2017
'High-throughput Field Phenotyping
– Plant Breeding in the Age of Gadgets and Big Data'

José Luis Araus

UNIVERSITAT DE BARCELONA

Outline

Phenotyping

- A bottleneck for breeding
- Current challenges
- Identifying the traits
- Selecting the tools for field phenotyping
- Effective and expensive are not synonyms
- Platforms
- More than traits, tools and platforms

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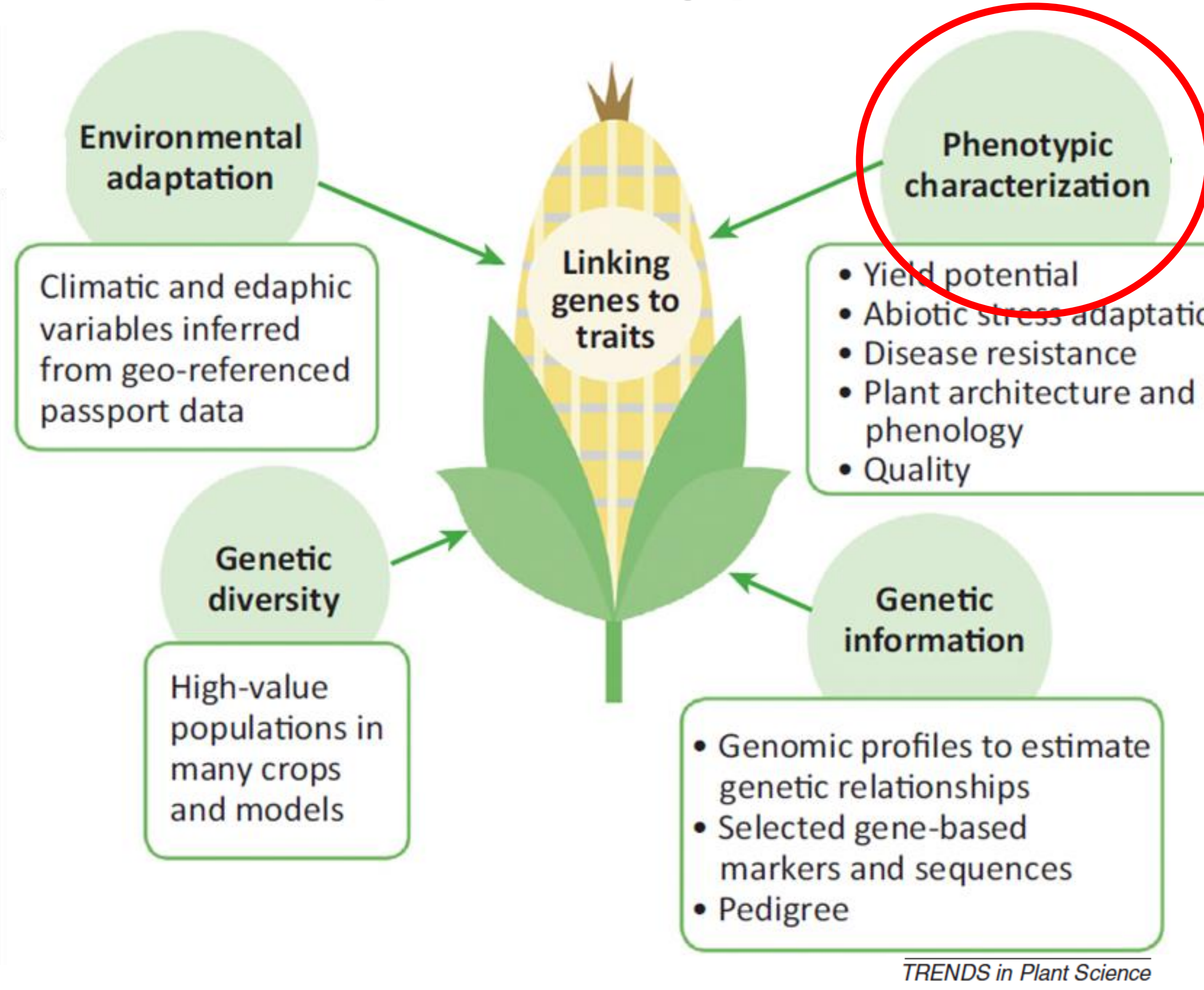
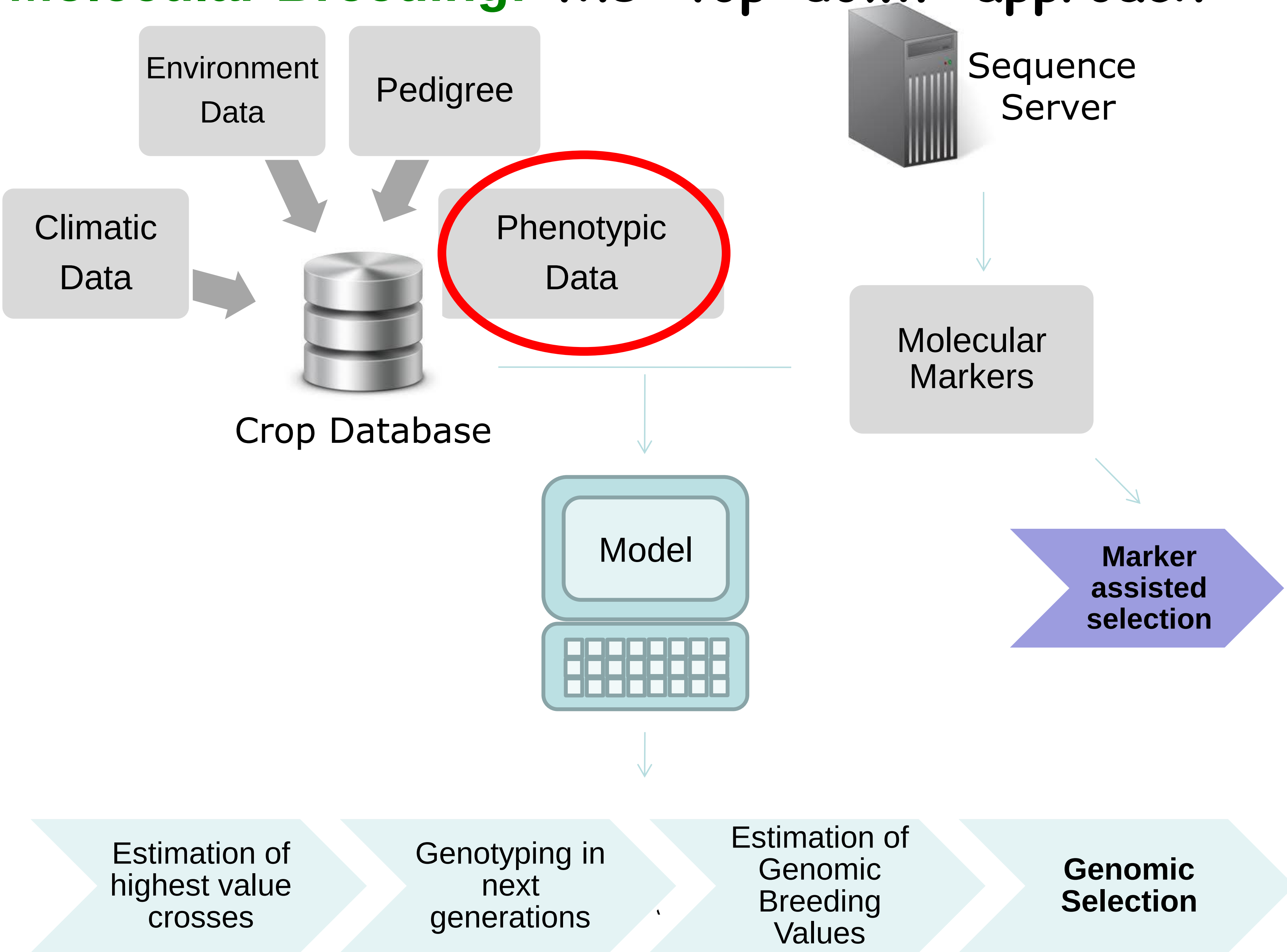
Crop breeding pillars



Araus and Cairns 2014 Trends Plant Sci.

Molecular Breeding: the "top-down" approach



Molecular Breeding
The “bottom-up” approach

COMPLEX

Top (phenotype)

Bottom (genes)

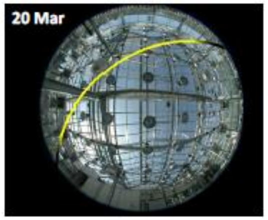
SIMPLE

Adapted from Roberto Tuberosa

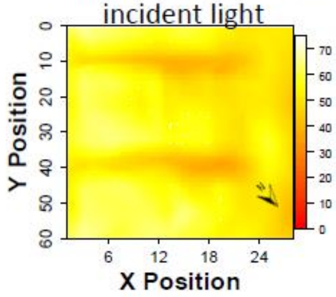
Phenotyping – still a bottleneck

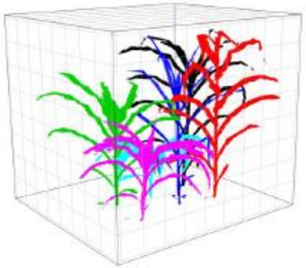
Controlled Environments

Daily sun paths



Spatial distribution of incident light





3D plant reconstruction

Virtual canopy scene of 1680 plants in the glasshouse

Cabrera-Bosquet et al. 2016 *New Phytologist*

Field

The world's first Field Scanalyzer is up and running at Rothamsted Research

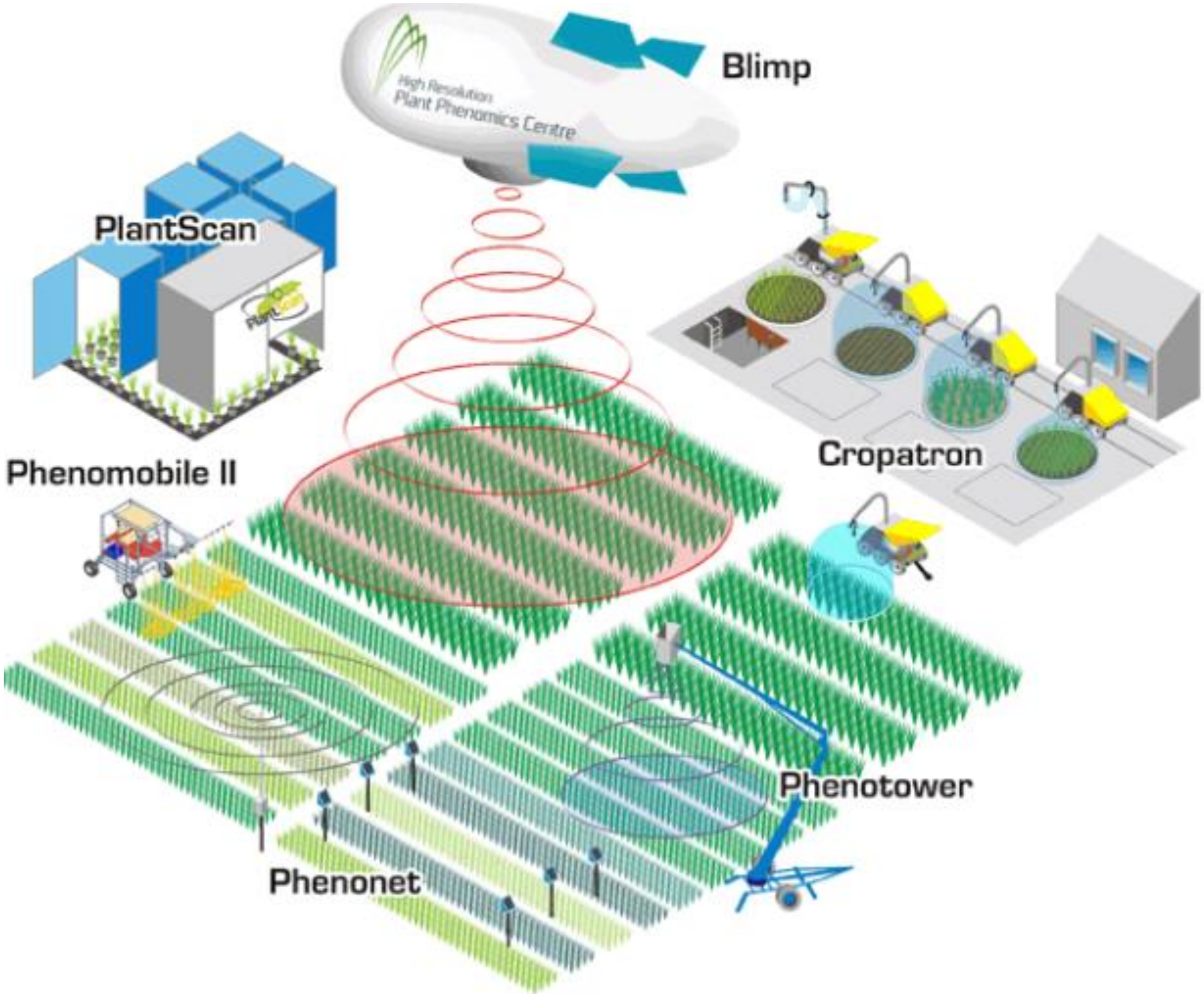
A unique facility for field phenotyping has been officially launched at Rothamsted Research.

Lemmatech, Montes et al 2011, FCR,;

Romano *et al.* 2012 Comp. Elect. Agric.

HRPPC Phenomics Technology

The High Resolution Plant Phenomics Centre (HRPPC) located in Canberra at CSIRO Plant Industry and the Australian National University is developing next generation research tools to probe plant function and performance, under controlled conditions from growth cabinets to the field. These new technologies include the **Phenonet**, **Phenomobile**, **Phenotower**, **Tethered Blimp**, **Cropatron** and the **PlantScan**.



Australian Plant Phenomics Facility

Plant phenotyping is getting increasing attention

ONE example for a national phenotyping platform

DPPN – German Plant Phenotyping Network

Deutsches Pflanzen Phänotypisierungs-Netzwerk

DPPN

Traits	Sensors	Positioning Systems	Experiments	Environmental Sensors
Roots →	Shovelomics / rhizotrons	Fixed Platforms	FACE infrastructure	Atmospheric / Soil parameters
Structure →	Stereo / structured light / LIDAR	**FieldLIFT** / Semi-fixes platforms	Common Experiment	
Water →	Active & passive thermography	**FieldCOP**/ Mobile Platforms		
	NIR spectroscopy	**FieldBEE** and **FieldSHIP**/ Octocopter and Zeppelin		
Photosynthesis →	PAMs / LIFT / sun-induced fluorescence			

Plant phenotyping is developing dynamically

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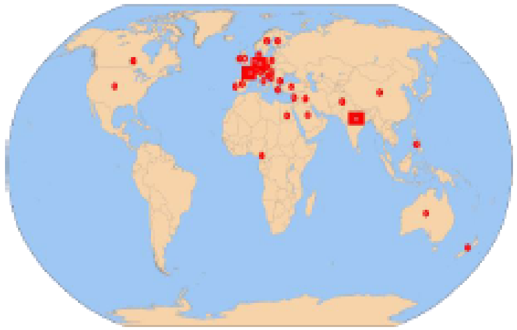
Demand and Next Challeneges

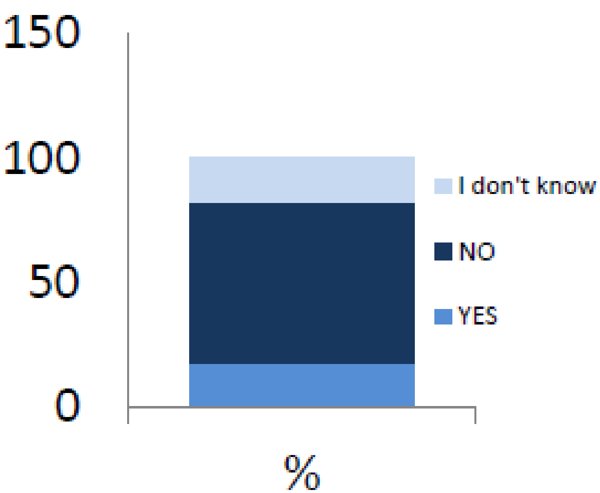
Plant Phenotyping Survey

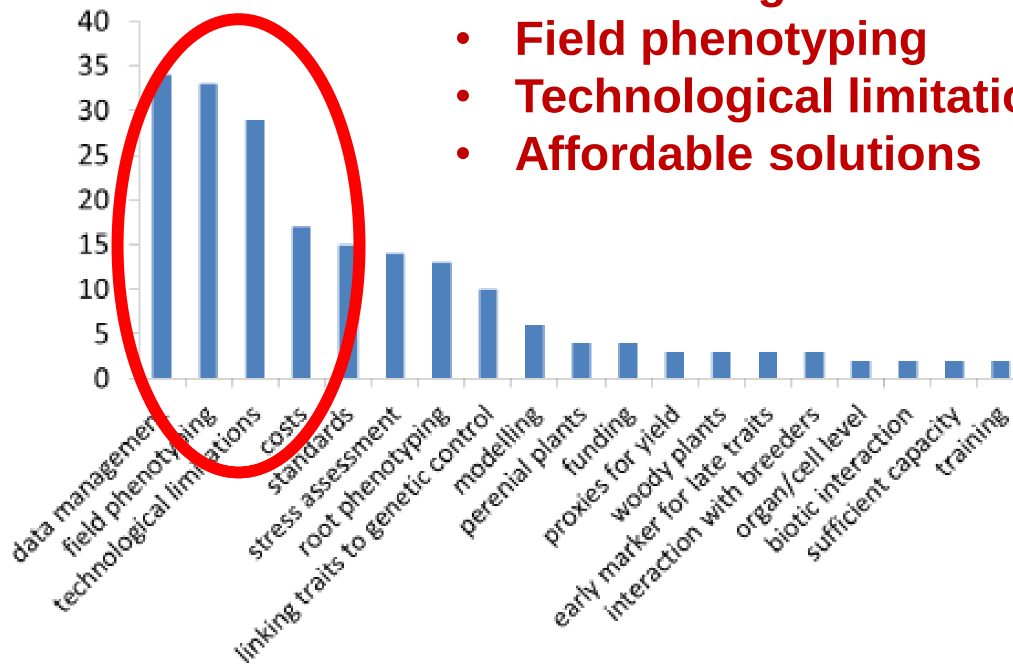


197 participants from 38 countries

Some results

Is there enough capacity?





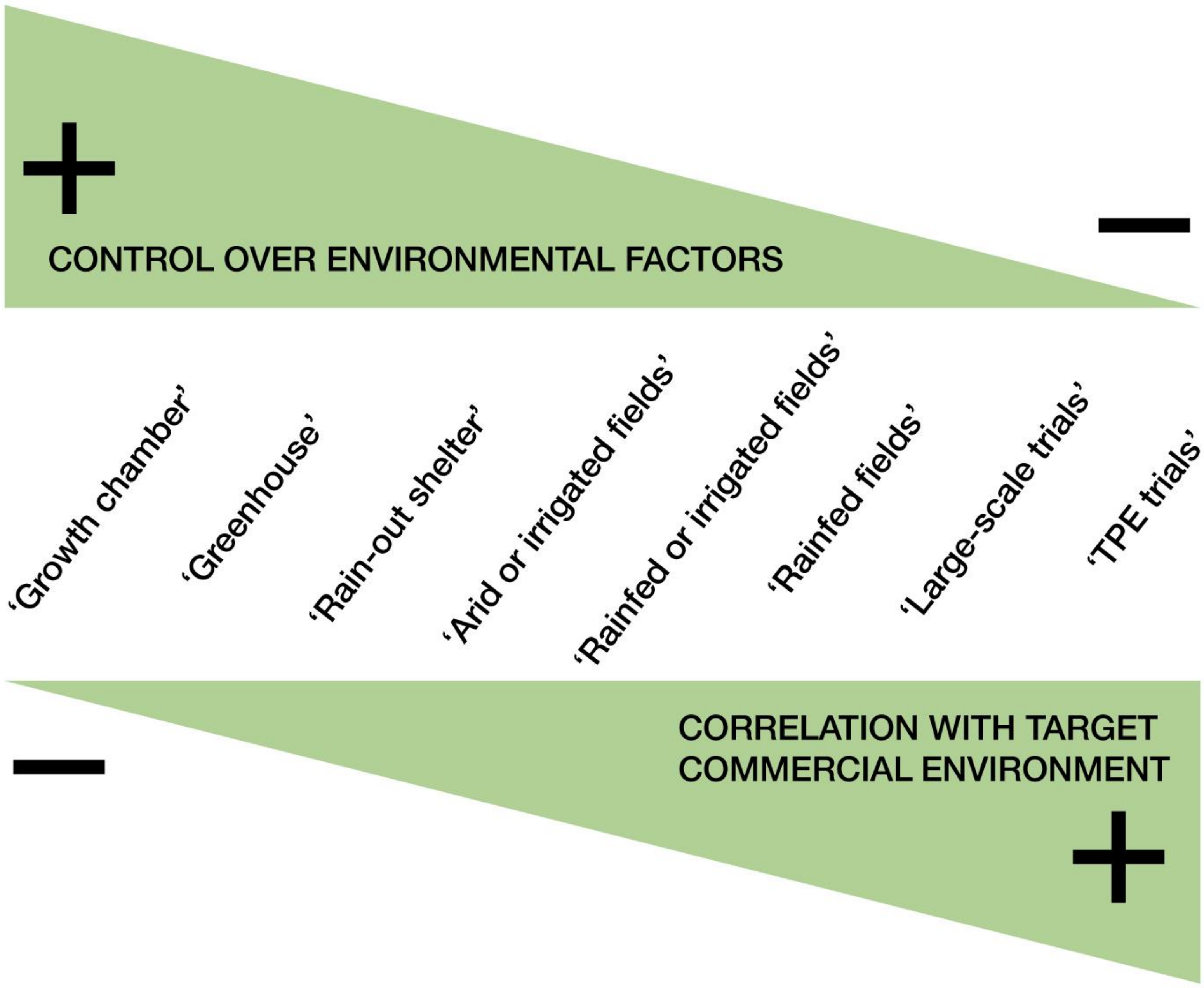
What are the next challenges?

- **Data management**
- **Field phenotyping**
- **Technological limitations**
- **Affordable solutions**

Phenotyping in Breeding - Challenges

- Align phenotyping in controlled environment with targets for field phenotyping
- Increase throughput
- Systems adapted to breeding phases
- Appropriate level of resolution
- Low(er) cost, mobile solutions

- Information management
  - Infrastructure and tools adapted to data types and volumes - automation
  - User friendly data management and analysis tools
  - Connection to other data systems - Data integration



After Passioura (2006) Funct. Plant Biol. 33,

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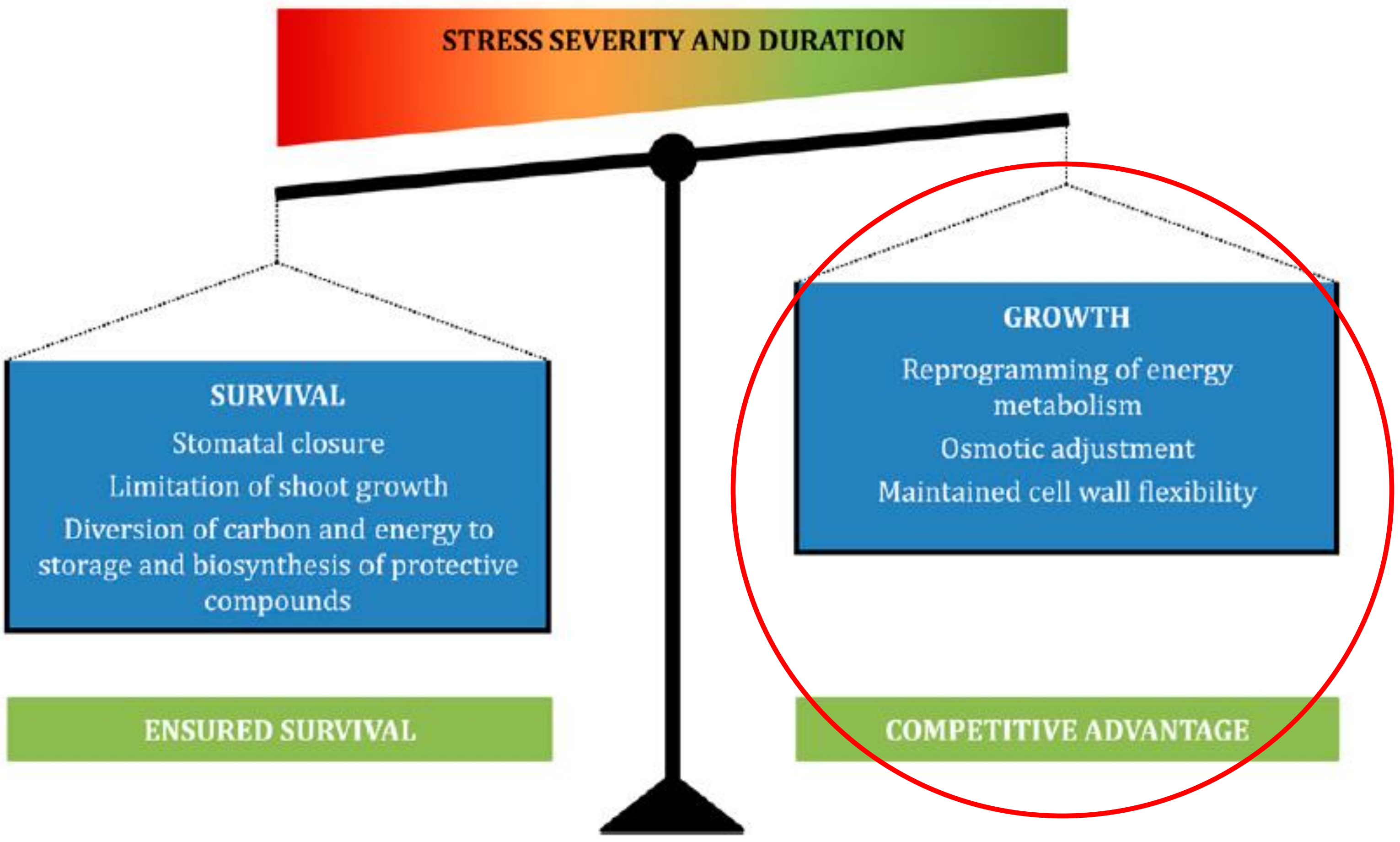


Plant Physiol. Vol. 162, 2013

Physiological yield Components

<u>General Determinant</u>

Yield = $\boxed{IR \times AR} \times \boxed{PE} \times \boxed{HI}$

- IR, Incident Radiation
- AR, Absorbed Radiation
- PE, Photosynthetic Efficiency
- HI, Harvest Index

Radiation uptake

Radiation use efficiency

Harvest Index

<u>In Water-limiting Conditions</u> (*Passioura 1977*)

Yield = $\boxed{W} \times \boxed{WUE} \times \boxed{HI}$

- W, Water Used
- WUE, Water Use Efficiency
- HI, Harvest Index

Water use

Water use efficiency

Harvest Index

Maize

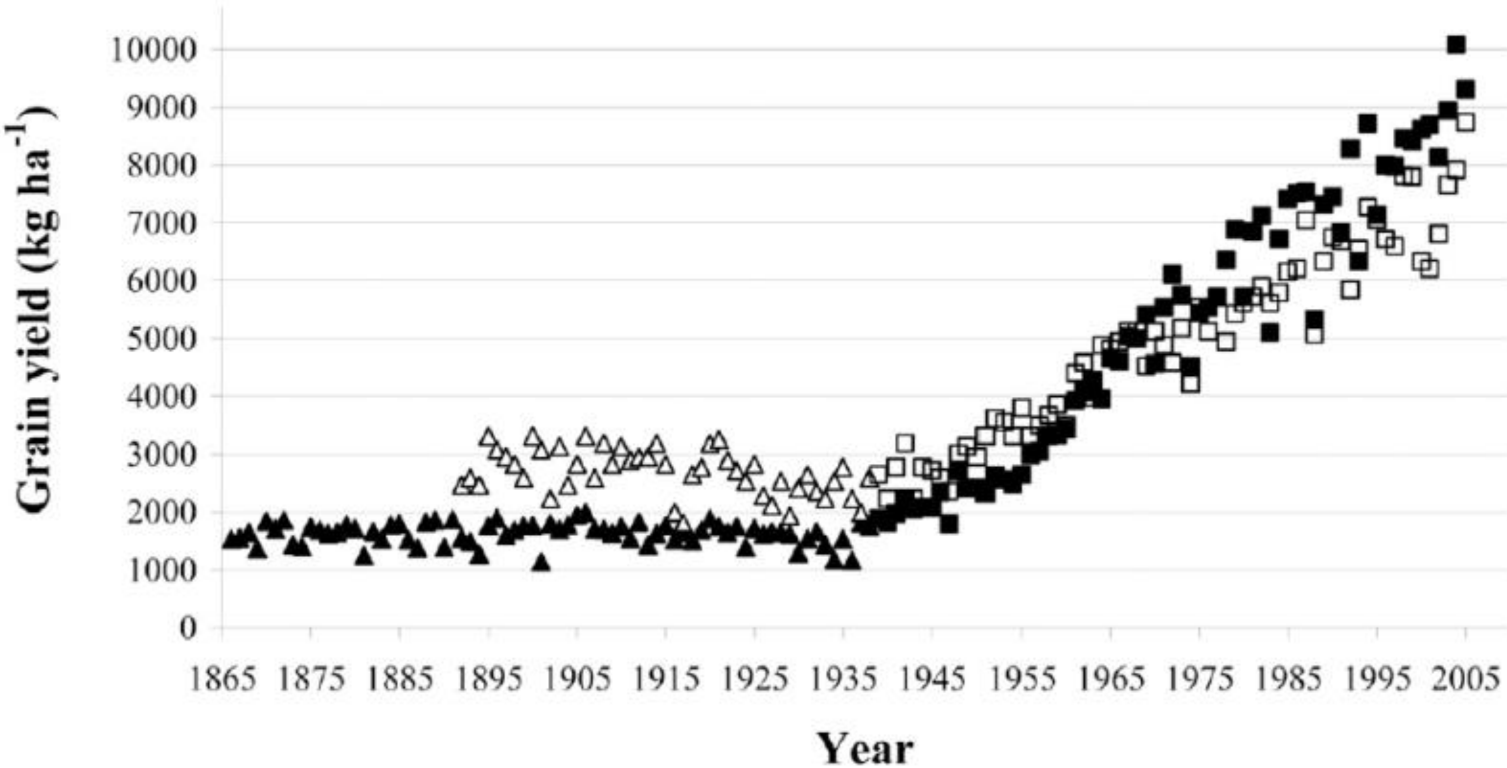
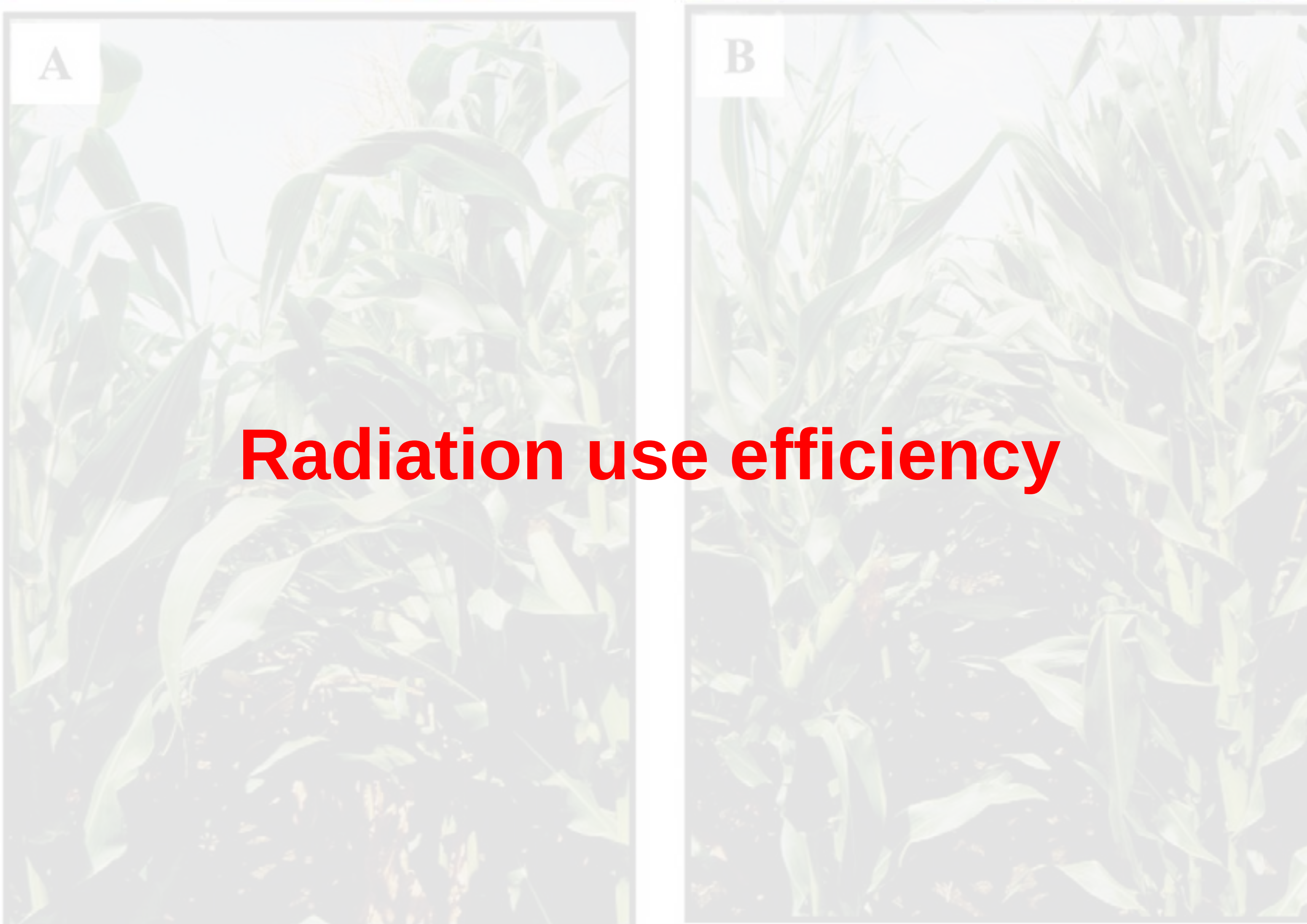


Figure 1. Average U.S. (1865–2005) and Canadian (1892–2005) maize yields in kilograms per hectare (15.5% moisture), 1866 to 1938 (pre-hybrid era; ▲– US yields, △– Canadian yields), 1939 to 2005 (hybrid era; ■– US yields, □– Canadian yields). Data compiled by the USDA and Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA).

Lee and Tollenaar 2007 Crop Science

More erect leaves in maize

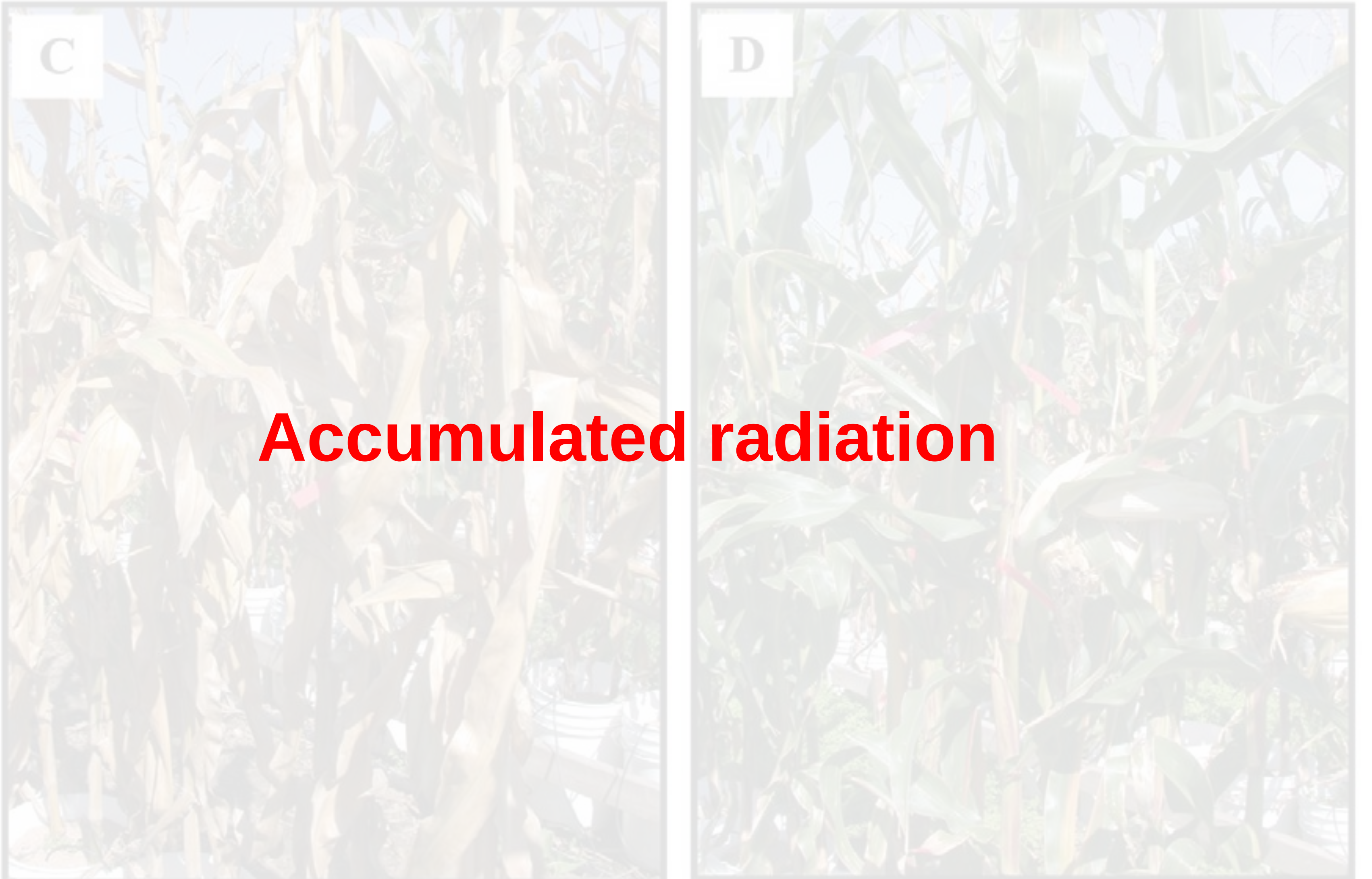


Radiation use efficiency

1930s 1990s

Lee and Tollenaar 2007, *Crop Sci*

Stay-green in maize



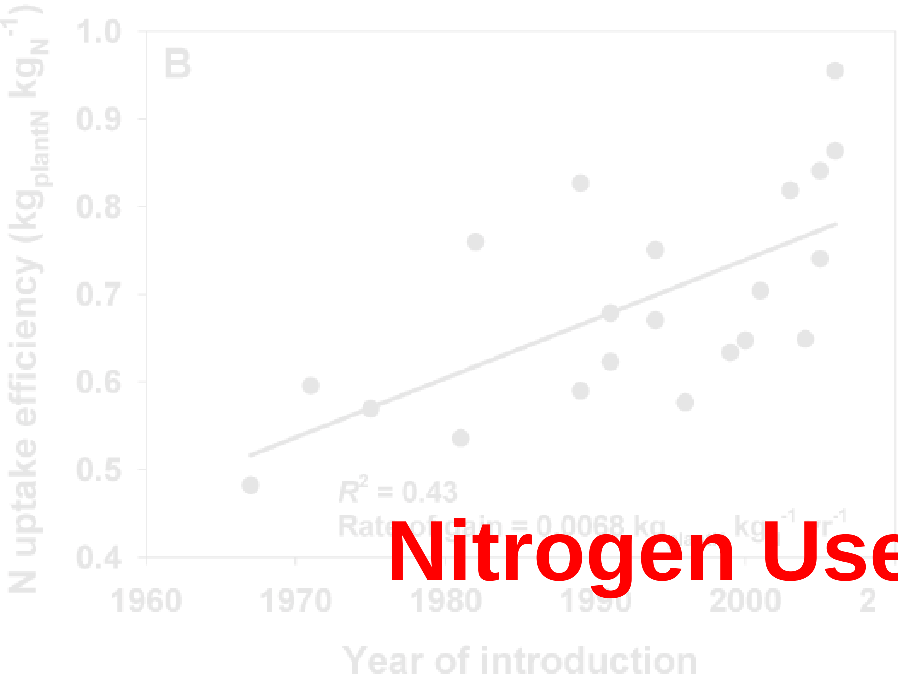
Accumulated radiation

1930s

1990s

Lee and Tollenaar 2007, *Crop Sci*

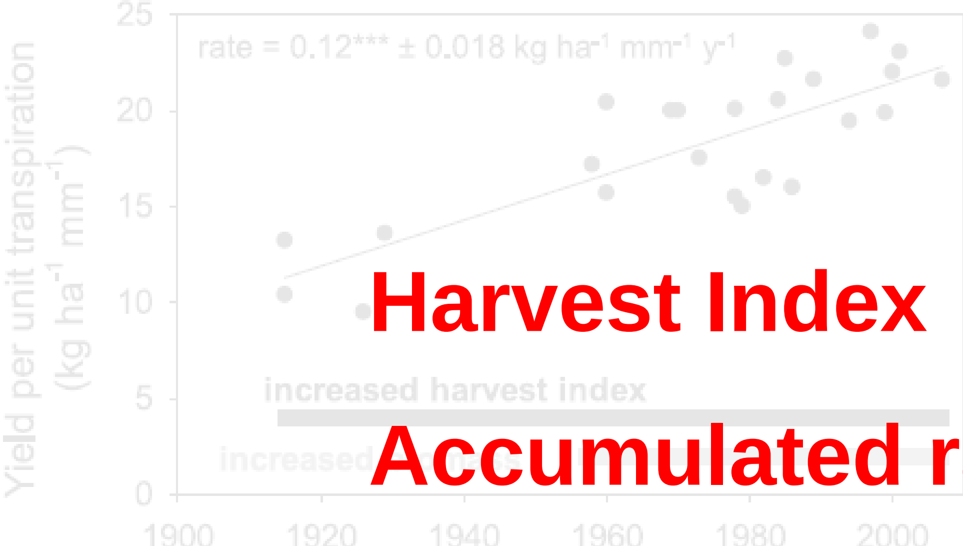
Stay-green – higher planting density



Nitrogen Use Efficiency

Haegele et al. 2013, *Crop Sci.*

Wheat



Harvest Index

Accumulated radiation

Water use efficiency

tr[illegible]	annual rate of change
anthesis biomass (kg ha^{-1})	24 ± 7.6*
maturity biomass (kg ha^{-1})	19 ± 5.7*
radiation use efficiency (g MJ^{-1})	0.012 ± 0.007***
shoot N (kg N ha^{-1})	0.40 ± 0.076***
shoot N per unit soil N (unitless)	0.002 ± 0.0004**
N nutrition index (unitless)	0.001 ± 0.00002***

Sadras and Richards 2014, *J. Exp. Bot.*

higher stomatal conductance - transpiration

TABLE 1
Inadvertent increase of stomatal conductance (g_s) and net assimilation rate (A) overtime by plant breeders in different crop species as documented by historical series field experiments conducted in different world growing regions for irrigated (I) and rainfed (R) conditions

Crop	Region/country	History Span	Number varieties	Years of Testing	g_s (in mmol H_2O m^{-2} s^{-1})	Correlations[1]					References
						Yield/RO	g_s/RO	g_s/yield	A/RO	A/yield	
Spring wheat -I	Sonora, Mexico	1962–1988	8	3	400–630	0.92***	0.89**	0.93***	0.25	0.02	Lu *et al.*, 1998
Spring wheat - I	California, USA	NA	13	1	NA	NA	NA	0.64**	NA	0.18**	Lu *et al.*, 1998
Durum wheat - I	Sonora, Mexico	1967–1989	7	2	NA	NA	NA	0.79**	NA	0.52	Fisher, unpubl.[2]
Spring wheat - I	Sonora, Mexico	1962–1988	8	2	345–570	NA	NA	0.89***	NA	0.85**	Fisher *et al.*, 1998
Pima cotton - I	Arizona, USA	1949–1992	8	2	690–860	0.94***	0.83**	0.92***	0.46	0.49	Lu *et al.*, 1998
Soybean - R	Ontario, Canada	1934–1992	14	4	1.25–1.55[3]	0.78**	0.67**	0.57*	0.84**	0.57*	Morrison *et al.*, 1999
Winter wheat - I	Beijing, China	1945–1995	18	1	400–1300	0.65*	NA	0.67**	NA	0.61*	Jiang *et al.*, 2003
Rice – I[4, 5, 6]	Tohoku, Japan	1893–1991	10	[illegible]	600–[illegible]	[illegible].82**	0.35	NA	0.54	NA	Zhang and Kokubun, 2004
Durum wheat - I	Foggia, Italy	1900–2000	14	2	250–425	0.86***	0.27	NA	0.56**	NA	De Vita *et al.*, 2007
Durum wheat - I	Sardinia, Italy	1900–2000	20	2	2.0–2.3[7]	NA	−0.54**	−0.69***	NA	NA	Giunta *et al.*, 2008
Winter wheat - R	Fars, Iran	1940–2000	15	2	150–250	0.88**	0.66**	0.63*	0.45	0.48	Miri, 2009
Winter wheat - R[8]	Henan, China	1981–2008	18	2	215–389	0.69**	NA	0.69**	0.42**	0.65**	Zheng *et al.*, 2011
Winter wheat - I	Shandong, China	1969–2006	15	3	NA	NA	NA	NA	NA	0.69**	Xiao *et al.*, 2012
Spring wheat - I[5]	Chillan, Chile	1920–2008	14	2	420–830	0.70**	0.51**	0.29*	NA	NA	del Pozo *et al.*,2014
Tomato –I[5,6]	California, USA	1930–2000	8	1	800–1200	0.28	0.50	0.33	0.56	0.33	Barrios-M and Jackson, 2014
Winter wheat - I	Shaanxi, China	1940s–2010s	8	2	NA	0.85**	NA	NA	0.77**	NA	Sun *et al.*, 2014
Winter wheat - R	Shaanxi, China	1940s–2010s	8	2	NA	0.67*	NA	NA	0.67*	NA	Sun *et al.*, 2014
Spring wheat - R	Parana, Brazil	1940–2009	10	2	475–630	NA	NA	0.83**	NA	0.88**	Beche *et al.*, 2014

Water use

The historical timeframe (history span) of these studies covers all years of varietal release (release order or RO) for production by respective breeding programs.
[1]Coefficient of correlation between yield, release order, g_s and A are indicated with (*), (**), and (***) symbols indicating levels of significance of alpha = 0.05, 0.01, and 0.001, respectively; NA = No data available.
[2]Presented in Fisher and Edmeades (2010).
[3]Stomatal conductance expressed in cm/m^{-2}.
[4]Gas exchange measured three weeks after heading; data from high nitrogen treatment presented, only.
[5]Values of g_s estimated from published graph data.
[6]Correlation coefficients calculated from original published data.
[7]$\log_{10}$ of leaf resistance being used in this study with negative r values between leaf resistance vs. year of release or yield.
[8]Among two locations of historical series, gas exchanges parameters were measured only in Zhoukou, a rainfed location.

Roche 2015 *Critical Rev. Plant Sciences* 34

Effect of water stress / heat on Harvest Index

Crop yield depends

- Amount of resources captured (%RIxGLD, Ptrans)
- Efficiency on the use of resources (RUE, WUE)
- Dry matter partitioning (harvest index)

also of:

Agronomical yield components…

Table 3. The summary of cereal traits quantifiable with sensors mounted on field buggies and the primary effect contributing to yield.

Trait	Primary Effect	Sensor Technology
Canopy structure		
Leaf area index	RI	LiDAR, 2D and 3D RGB photogrammetry, ToF camera, spectral vegetation indices
Biomass	WUE/RUE	LiDAR, 2D and 3D RGB photogrammetry, ToF camera
Tillering	HI	LiDAR, 2D and 3D RGB photogrammetry, ToF camera
Canopy height	WUE/HI	LiDAR, 2D and 3D RGB photogrammetry, ToF camera
Awn presence	WUE/HI	LiDAR, 2D and 3D RGB photogrammetry, ToF camera
Leaf rolling	WUE/RI	LiDAR, 3D RGB photogrammetry and ToF camera
Leaf angle	RI	LiDAR, 3D RGB photogrammetry and ToF camera
Early vigour	WUE/WU	LiDAR, 2D RGB photogrammetry, spectral vegetation indices
Tissue damage	WU/RI	RGB camera, multi/hyperspectral camera
Leaf glaucousness/waxes	WUE/HI	Multi/hyperspectral camera
Pubescence	WUE/HI	Multi/hyperspectral camera
Grain fertility (number)	HI	Very high resolution RGB images
Function		
Water loss/stomatal control	WUE/WU	Thermal camera, infra-red temperature sensor
Photosynthesis	RUE	Chlorophyll fluorescence, LIFT, PRI, estimation from biomass accumulation (see above)
Phenology		
Stay green/senescence	HI/RI	LiDAR, multi/hyperspectral camera, thermal camera
Flowering date	HI	LiDAR, high resolution RGB images
Biochemistry		
Stem carbohydrates	HI	hyperspectral camera
Nutrient content (e.g., N)	NUE	Multi/hyperspectral camera
Carotenoids, xanthophylls, anthocyanins, water indices	WU/RI	Multi/hyperspectral camera

HI = harvest index; LIFT = laser-induced fluorescence transients; NUE = nitrogen-use efficiency; PRI = photochemical reflectance index; RGB = red, green and blue; RI = radiation interception; RUE = radiation-use efficiency; ToF = time of flight; WU = water-use; WUE = water-use efficiency.

Deery et al. 2014 *Agronomy*

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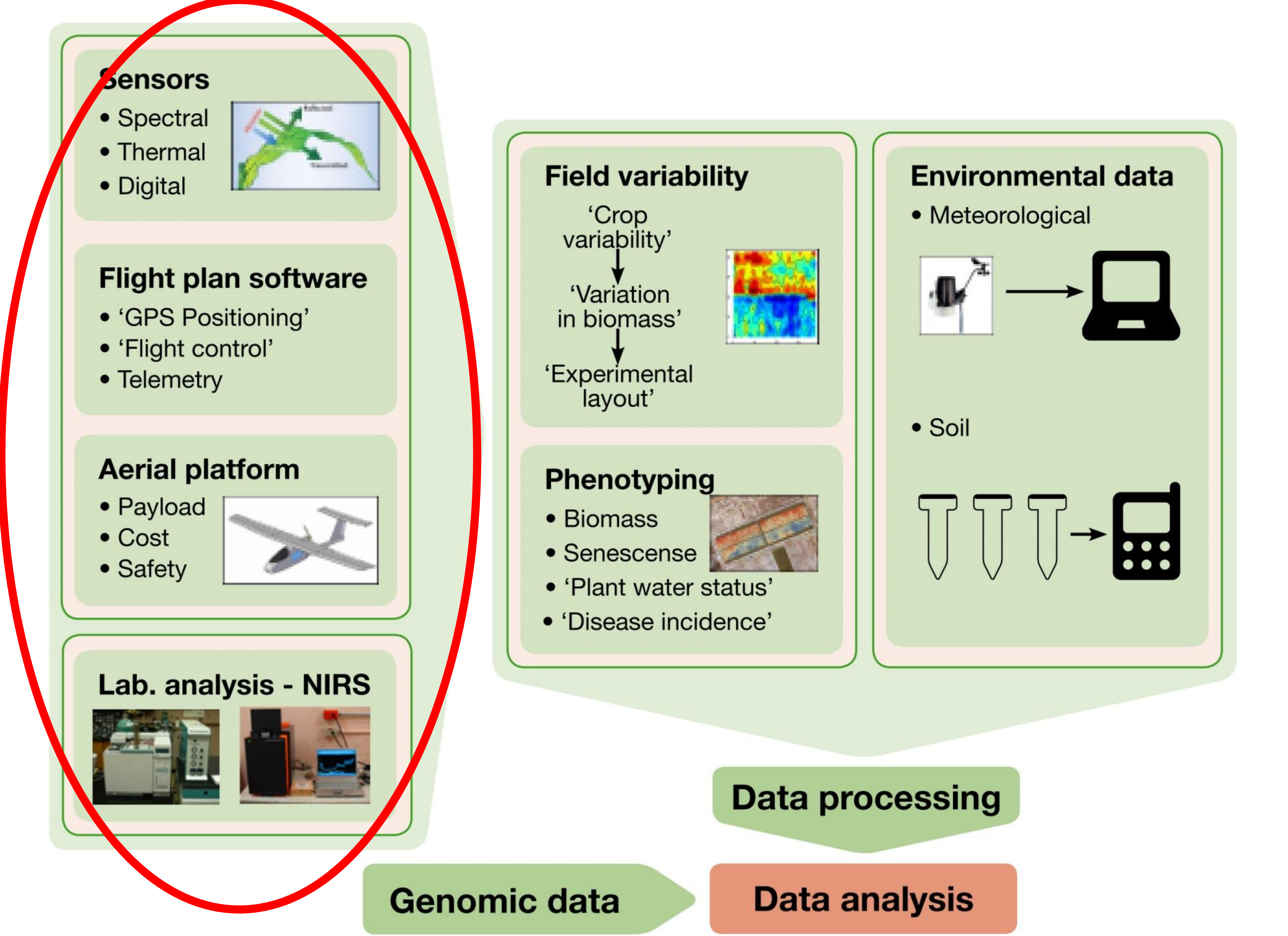
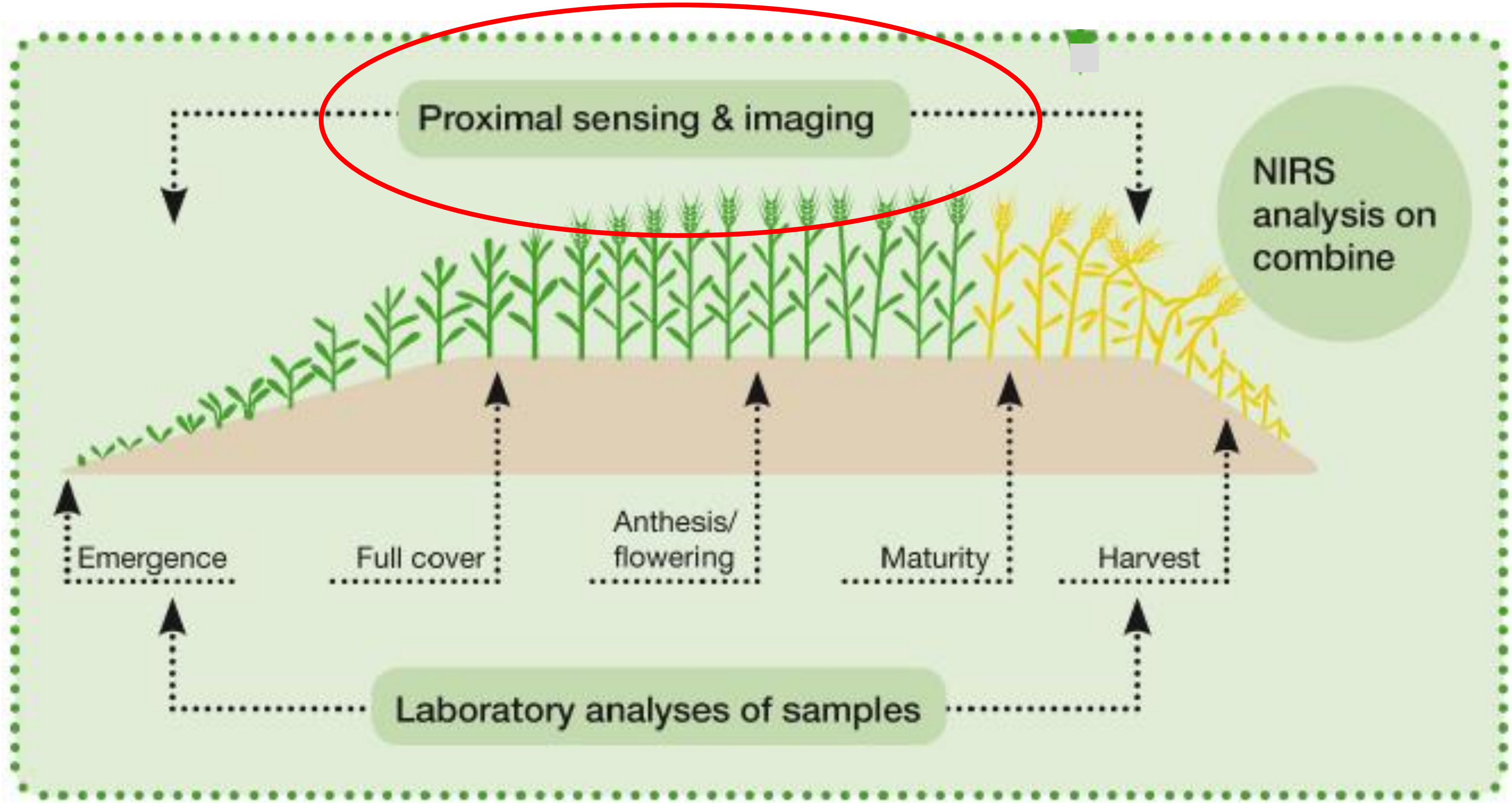


Sensors
• Spectral
• Thermal
• Digital
Flight plan software
• 'GPS Positioning'
• 'Flight control'
• Telemetry
Aerial platform
• Payload
• Cost
• Safety
Lab. analysis - NIRS
Field variability
'Crop variability'
'Variation in biomass'
'Experimental layout'
Phenotyping
• Biomass
• Senescense
• 'Plant water status'
• 'Disease incidence'
Environmental data
• Meteorological
• Soil
Data processing
Genomic data
Data analysis

Different categories of traits



Canopy senescence – visual score

Measurement:

- score from 0-10, divide the % of estimated total leaf area that is dead by 10
- initiation & rate of canopy senescence

1 (10%)

3 (30%)

5 (50%)

7 (70%)

9 (90%)

M. Banziger, CIMMYT

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Leaf rolling	WUE/RI	LiDAR, 3D RGB photogrammetry and ToF camera
Leaf angle	RI	LiDAR, 3D RGB photogrammetry and ToF camera
Early vigour	WUE/WU	LiDAR, 2D RGB photogrammetry, spectral vegetation indices
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Deery et al. 2014 *Agronomy*

Spectroradiometers

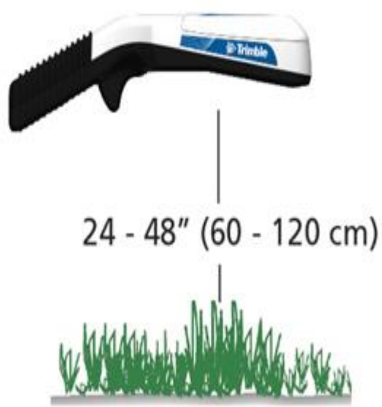
Spectroradiometers – active sensors

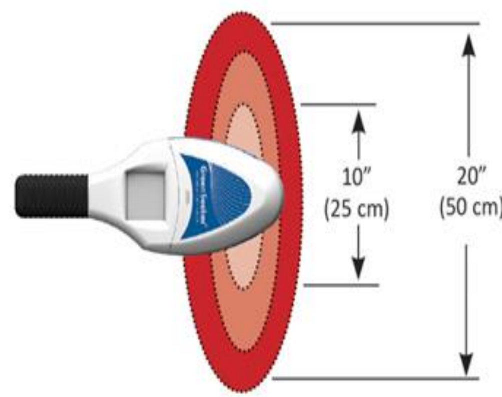
GreenSeeker | **SPAD**

New!

GreenSeeker®

HANDHELD CROP SENSOR





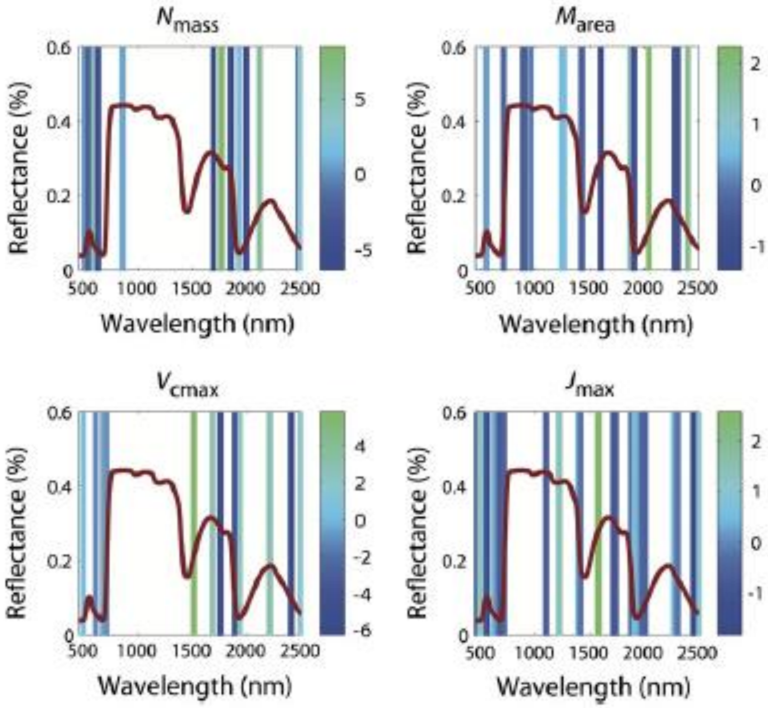
Spectroradiometers – pasive sensors

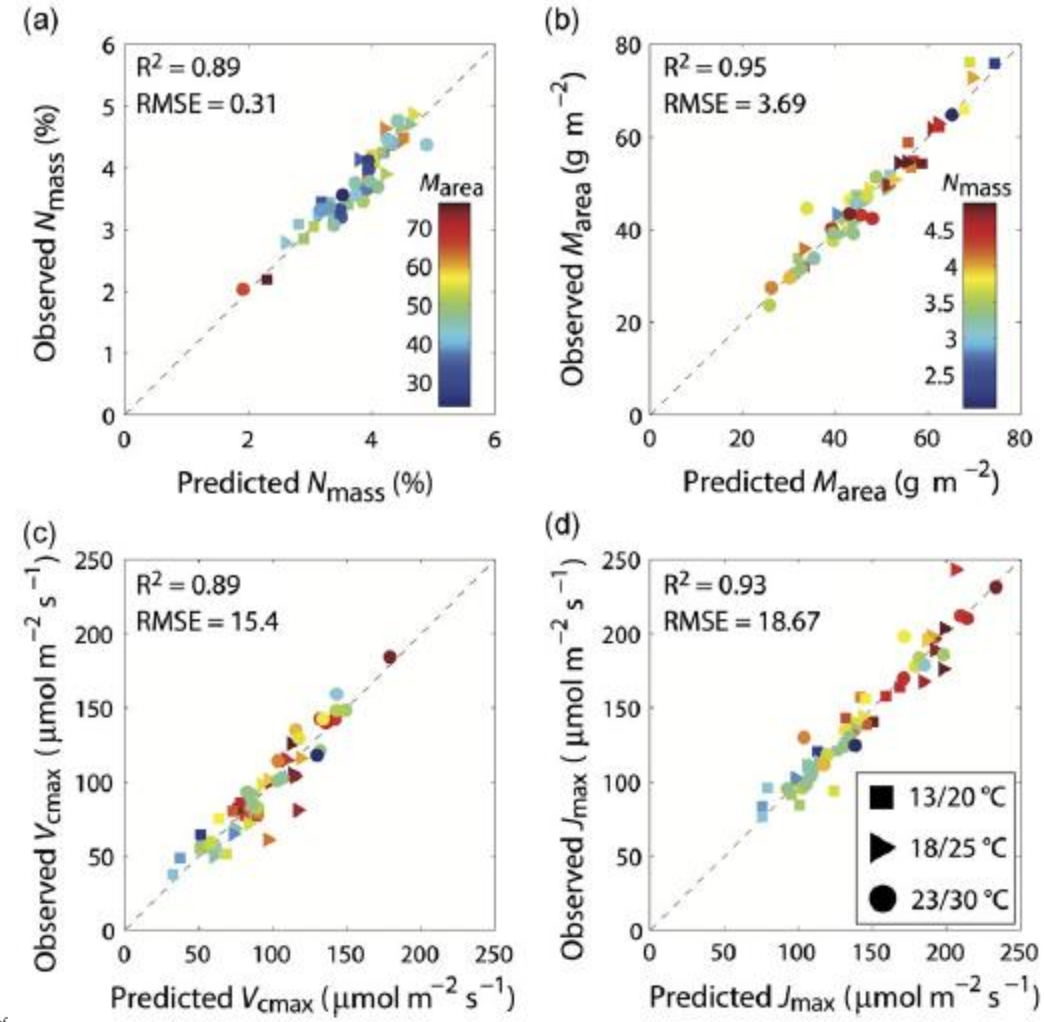
Full-range (λ 350 – 2500 nm) Vis/NIR Spectroradiometers

Spectroradiometrical Indices

Some indices for remote sensing of crop status.

Physiological parameter	Radiometric Index
Leaf area, [Chl], Green Biomass, etc.	$NDVI = \frac{R_{NIR} - R_{\mathrm{Re}\,d}}{R_{NIR} + R_{red}}$ $SR = R_{NIR} / R_{red}$ $SAVI = \frac{R_{NIR} - R_{\mathrm{Re}\,d}}{R_{NIR} + R_{red} + L}(1 + L)$ (where L=0.5 for most crops)
Chl degradation	$NPQI = \frac{R_{415} - R_{435}}{R_{415} + R_{435}}$
Car/Chl	$SIPI = \frac{R_{800} - R_{435}}{R_{415} + R_{435}}$
PRUE	$PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}$
Water Content	$WI = \frac{R_{900}}{R_{970}}$





Journal of Experimental Botany, Vol. 63, No. 1, pp. 489–502, 2012
doi:10.1093/jxb/err294 Advance Access publication 6 October, 2011
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany
www.jxb.oxfordjournals.org

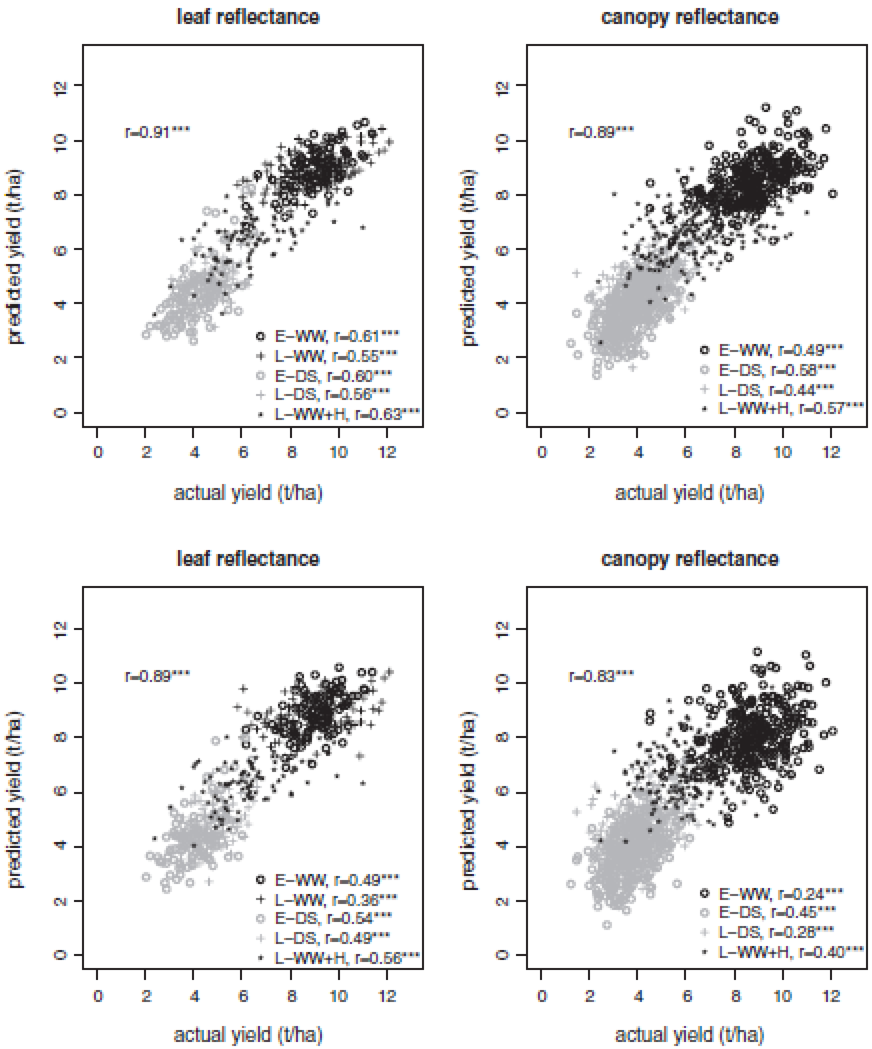
RESEARCH PAPER

Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature

Shawn P. Serbin*, Dylan N. Dillaway†, Eric L. Kruger and Philip A. Townsend

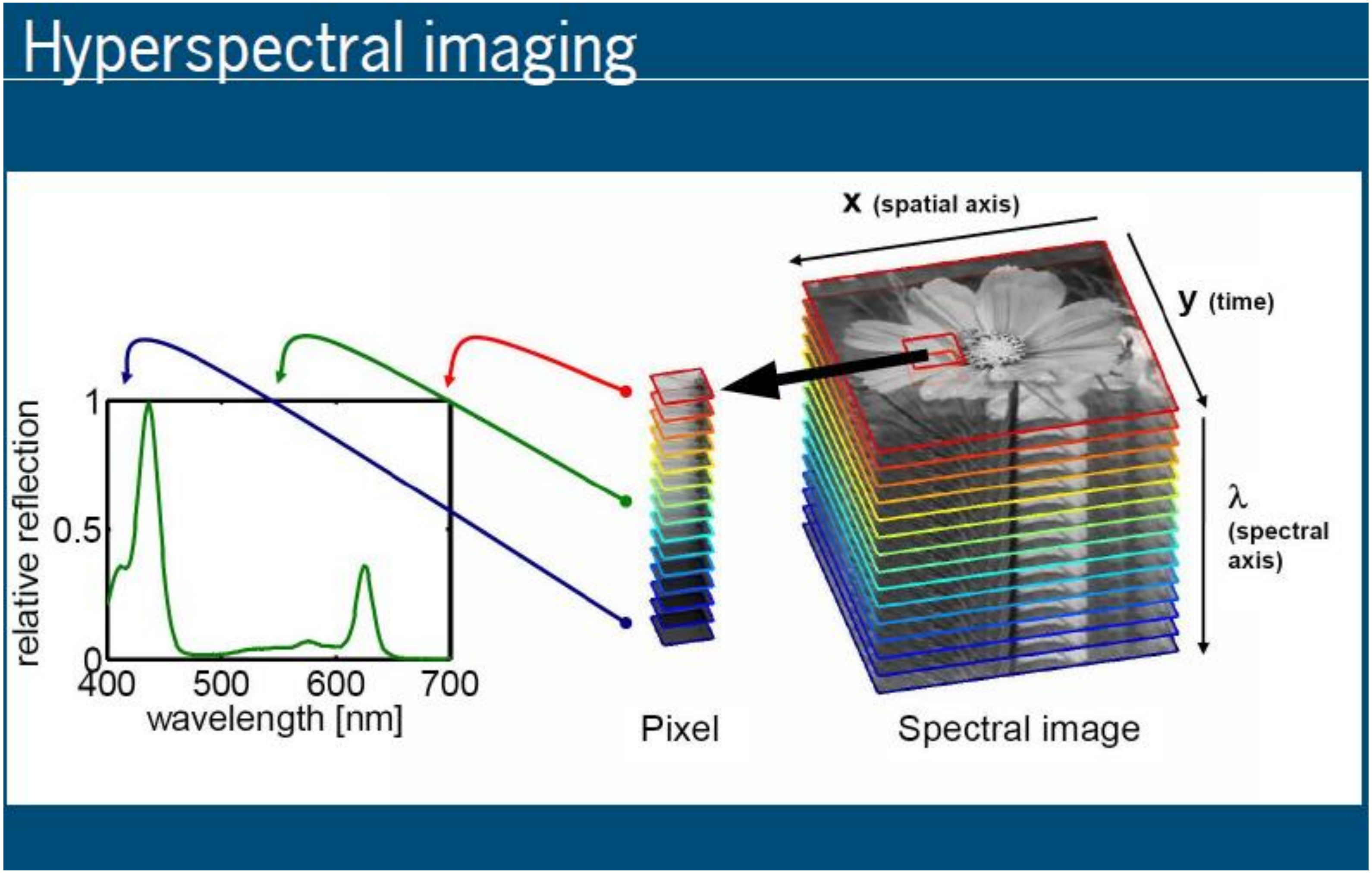
Direct spectroradiometrical assessment of GY in the field (using Full-range λ 350 – 2500 nm Vis/NIR Spectroradiometers)

V.S. Weber et al. / Field Crops Research xxx (2012) xxx–xxx

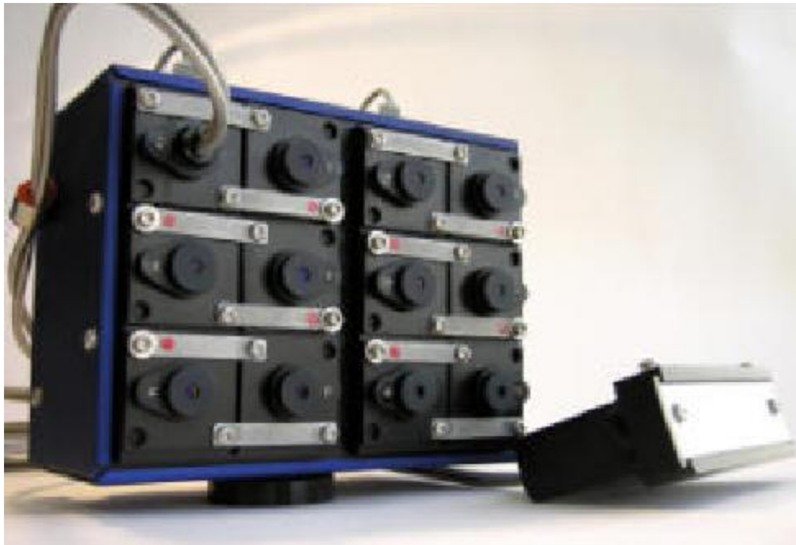


Weber *et al*. 2012 FCR 128: 82-90.

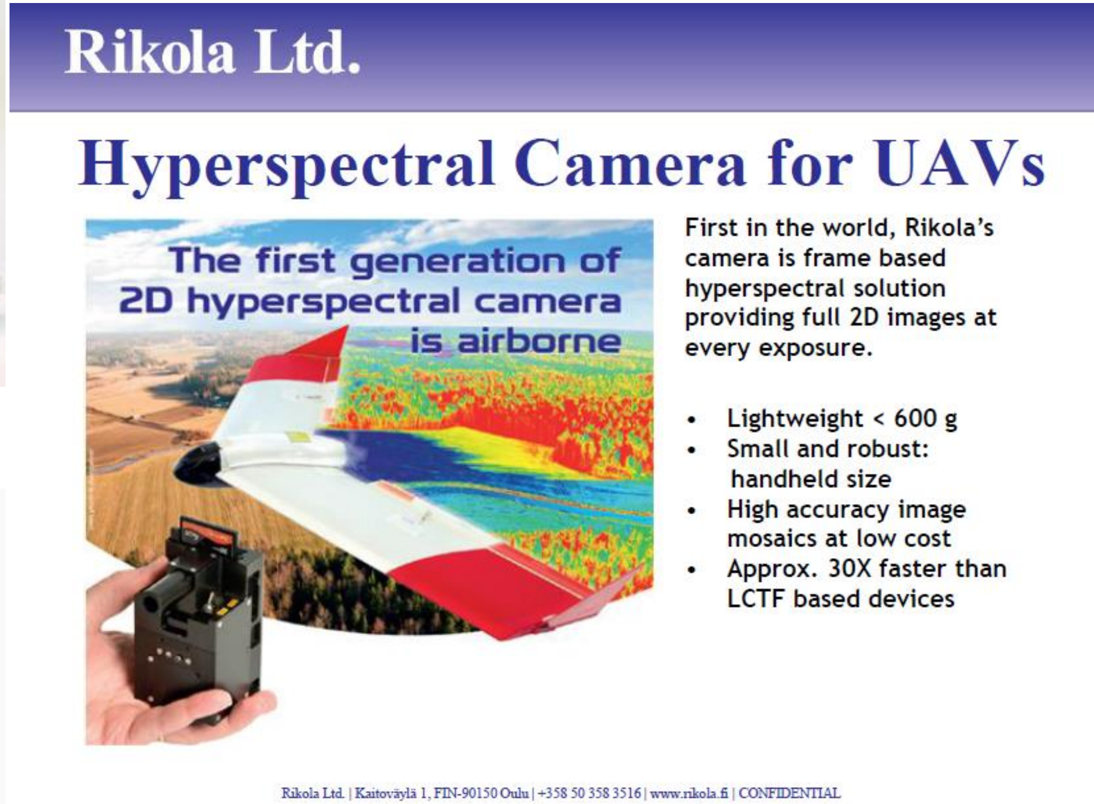
Multispectral – hyperspectral imaging



Mini Tetracam



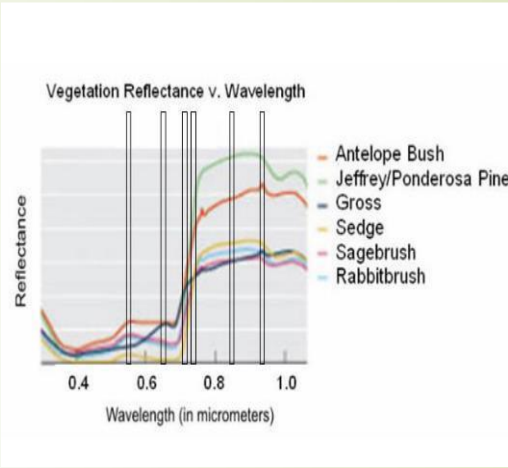
Micro Tetracam

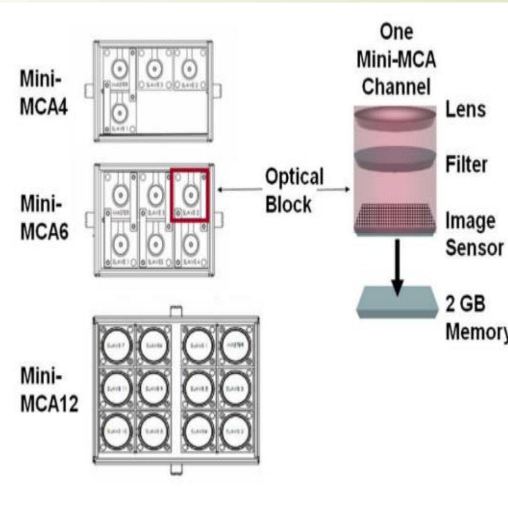


Tetracam mini-MCA 11+ILS

Tetracam mini-MCA 11+ILS Example Spectral Index Calculations

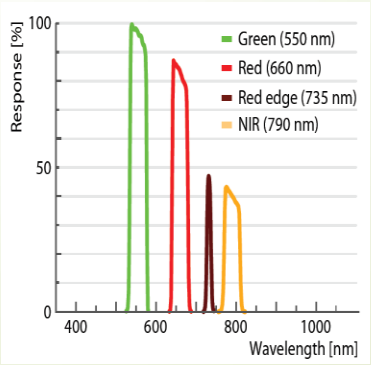
- NDVI = (R840 - R670) / (R840 + R670)
- PRI = (R550 - R570) / (R550 + 570)
- SAVI = (R840 - R670) / (R840 + R670 + L) · (1 + L)   (L=0.5)
- MCARI = [(R700 - R670) - 0.2 · (R700 - R550)] · (R700 / R670)
- WBI = (R900 / R950)
- RDVI = (R840 - R670) / ((R840 + R670)^1/2)
- EVI = 2.5 · ((R840 - R670)/(R840 + 6 · R670 - 7.5 · R450 + 1))
- ARI2 = R840 · [(1/R550) - (1/R700)]
- CRI2 = (1/R550) - (1/R700)
- WI = (R950 / R900)
- SR = (R900 / R670)
- SIPI = (R840 - R450) / (R840 - R670)
- NPCI = (R670 - R450) / (R670 + R450)





Other multispectral sensors available with 4-6 bands (at 500-1000 USD per sensor band)

- Tetracam 4 band ADC, ADC lite, and microMCA 4 or 6, customizable filters from 400-1000 nm, with or without ILS, optional thermal camera integration, and GPS units available separately.
- HiPhen AirPhen 6 sensor customizable bandwidth filters multispectral sensor with GPS and optional thermal camera integration.
- AIRINOV Multispec 4C NDVI-NDRE and NDVI-PRI 4 band sensors with GPS and ILS sensors integrated
- Parrot Sequoia 4 band + RGB sensor with integrate ILS, GPS and IMU

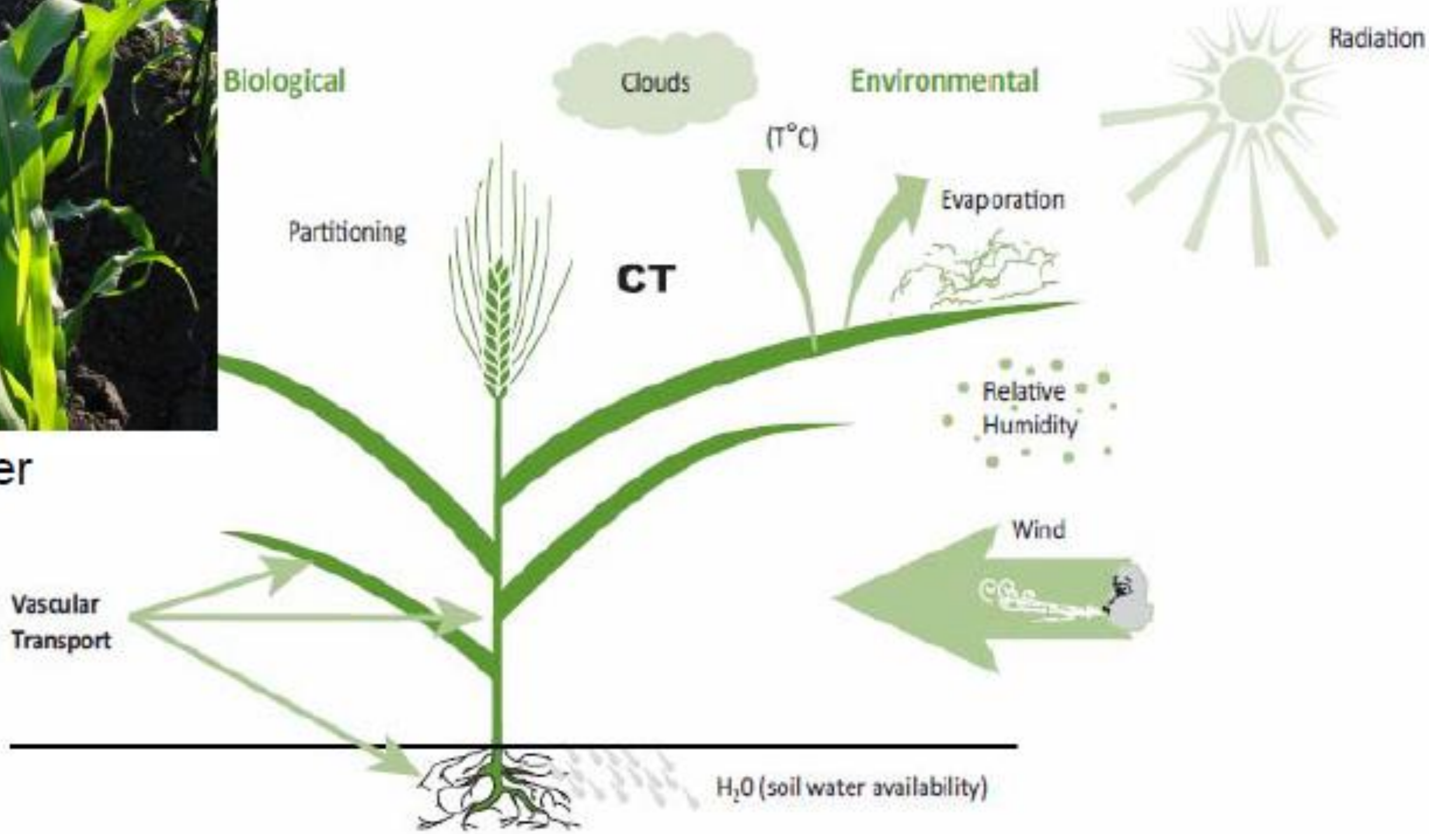


Thermal sensors

Transpiration as a cooling system: IR thermometry

IR Thermometer

With quiet air (i.e. limited air-cooling), differences of several degrees in fully irradiated leaves with changes in stomatal conductance



Reynolds, Pask & Mullan 2012

Figure 6.1. Biological (physiological) and environmental factors affecting canopy temperature (Adapted from Reynolds *et al.*, 2001).

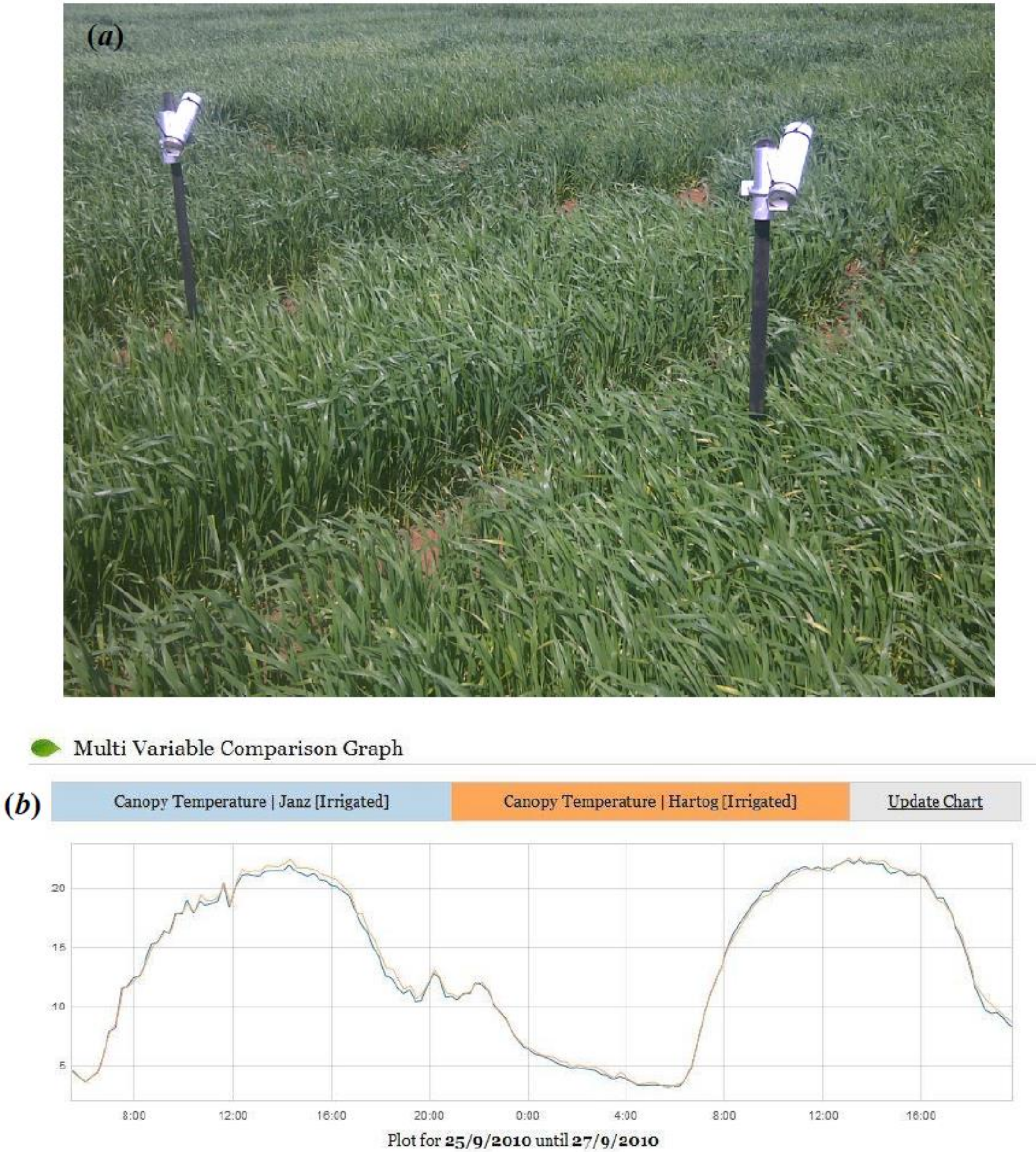


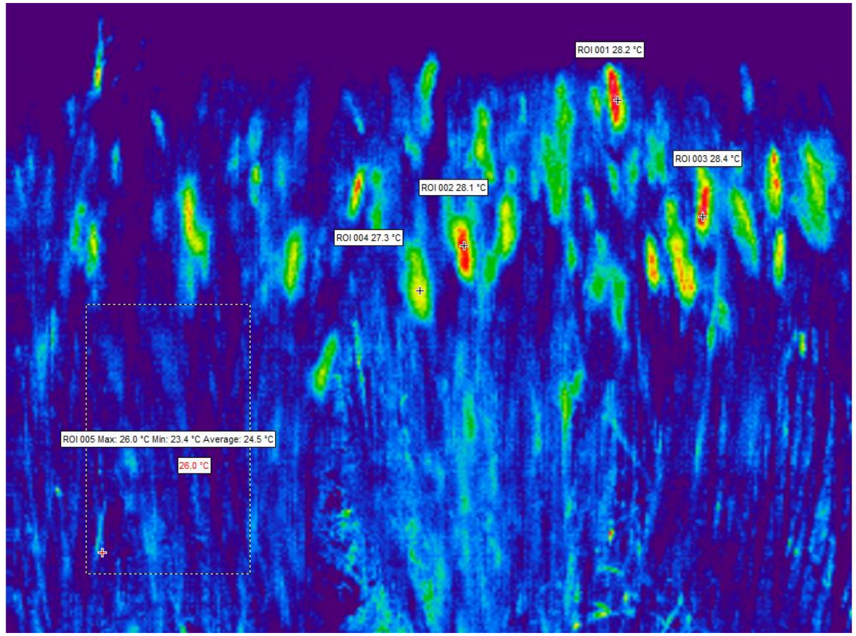
Fig. S2. Use of the Phenonet in monitoring of canopy temperature for multiple genotypes: (*a*) infra-red thermometers (Melexis®, 10 deg field of view) used for monitoring canopy temperature at the Yanco MEF; and (*b*) screen shot of the Phenonet visualisation and analysis system for near-real time recording of canopy temperature (here of wheat cultivars Janz (blue) and Hartog (orange) assessed under irrigated conditions).

Rebetzke et al. 2013 FPB 40: 1-13

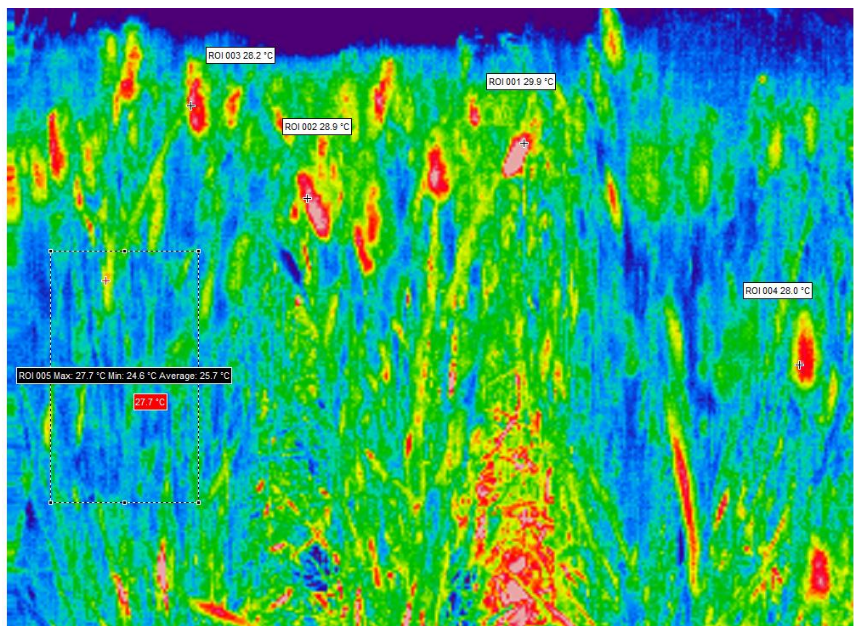
Thermal cameras

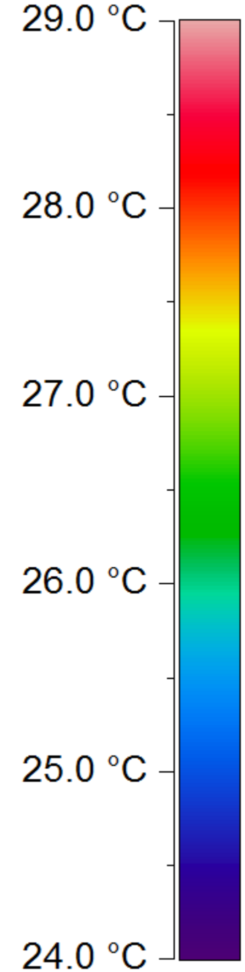
Ears/shoots

Supplemental irrigation

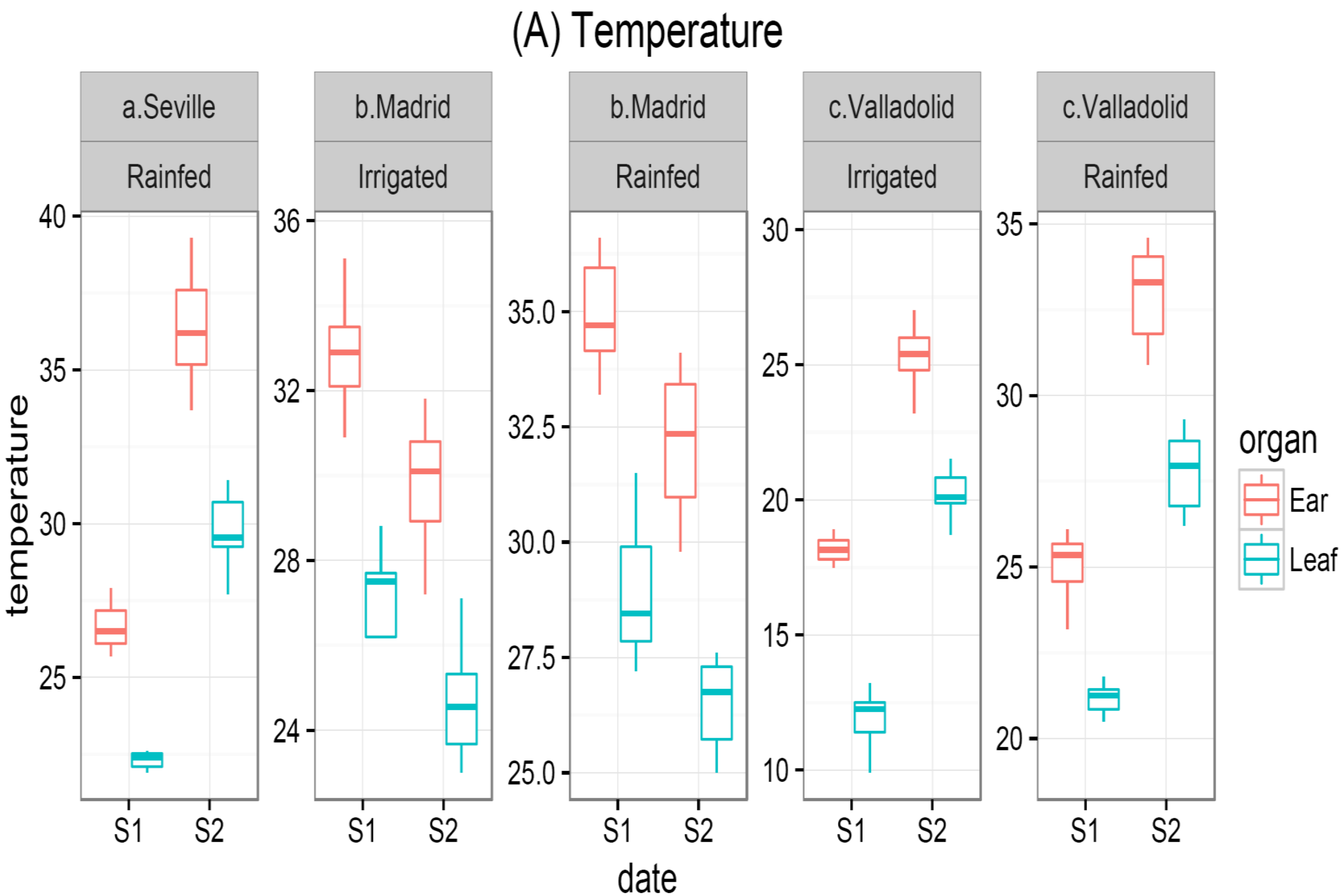


Rainfed





Temperature differences between flag leaves and ears under rain fed or irrigated conditions



Vicente et al., second revision at Plant Cell Physiology

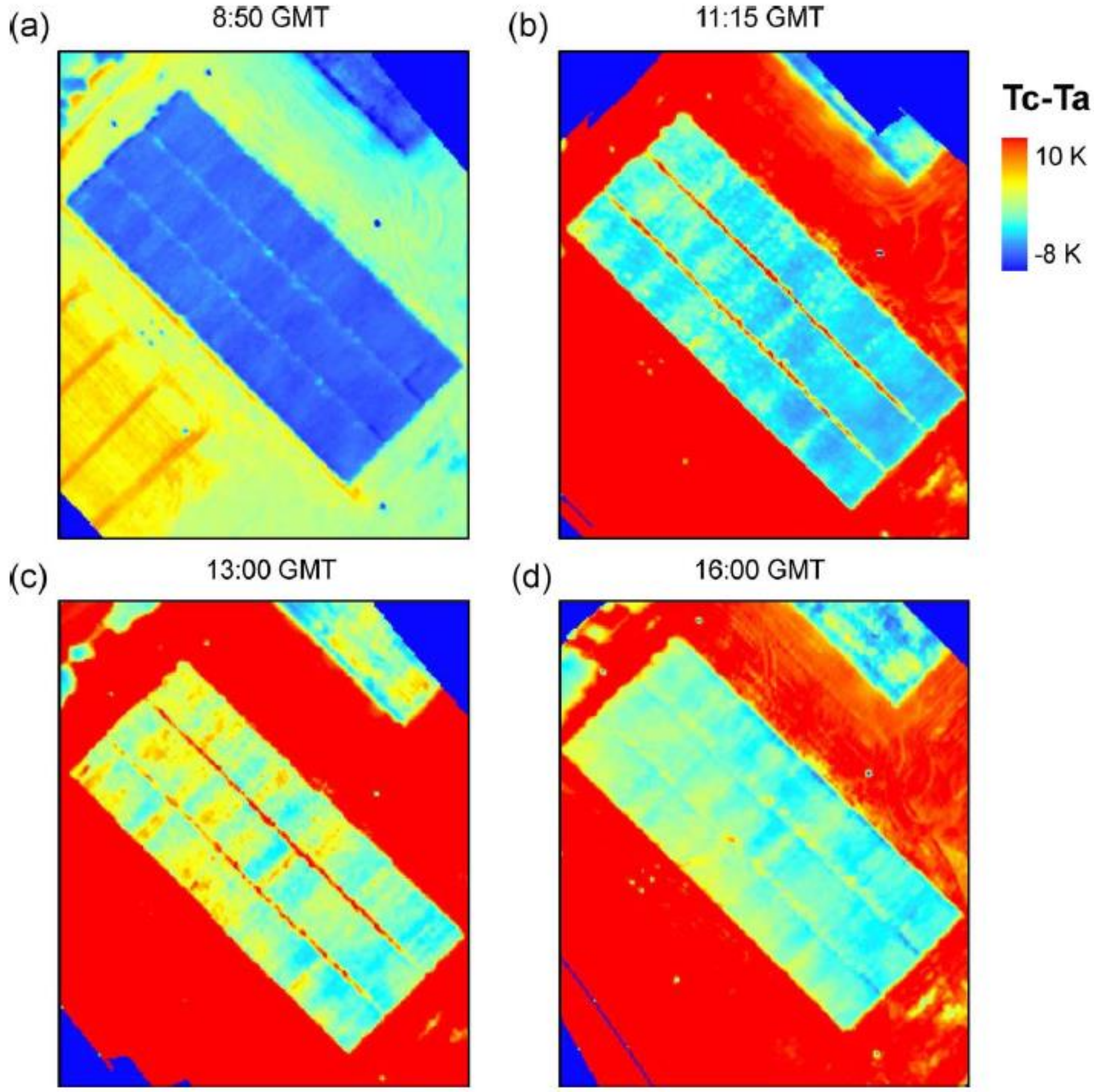


Fig. 14. Thermal images acquired over the corn field at 0.4-m pixel resolution showing the $T_c - T_a$ changes at four different times of day. The greatest thermal variability between corn variety plots is obtained at midday, continuing during the afternoon.

Bernie et al. 2009 IJRS 47**:** 722 - 738

New thermal image + RGB fusion sensors on the market

FLIR
Duo Pro R

TeAx is an international distributor for the FLIR Duo Pro R

Dual-Sensor Imaging
High resolution thermal and 4K color imagers integrated into a single powerful, convenient package.

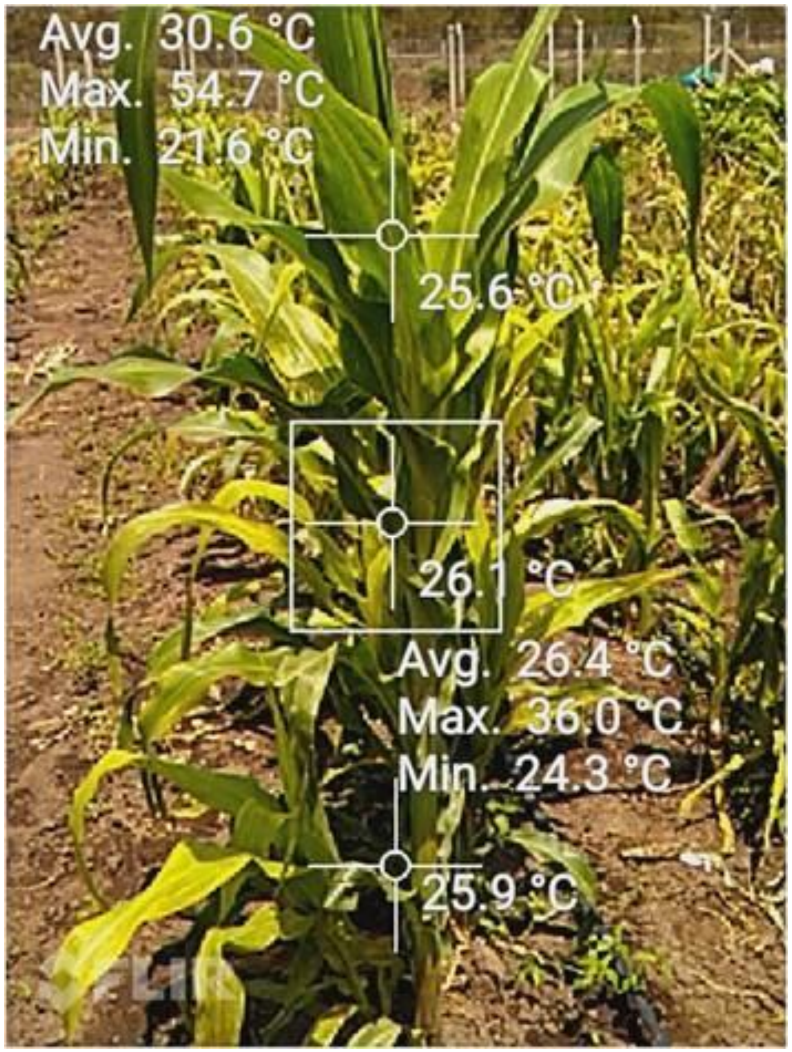
TEAX ThermalCapture
Fusion Zoom

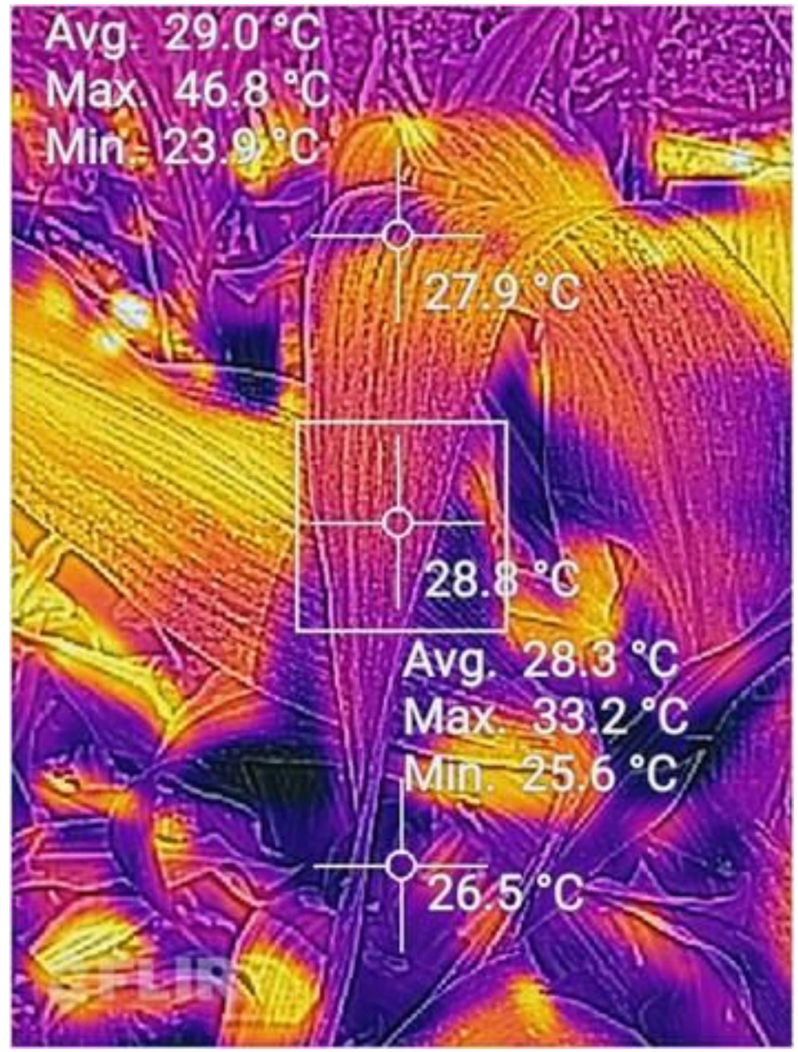
TC Fusion Zoom is being delivered to global clients already. Watch its powerful transparent overlay capacities in the video.

Perfectly aligned thermal and visual images
Stores full 14bit raw digital radiometric data
Optical vibration-compensation
10x times visual optical zoom


FLIR
CAT

Pictures taken from the camera using the thermal plus RGB fusion, thermal temp point measurements over RGB, and plain thermal camera modes





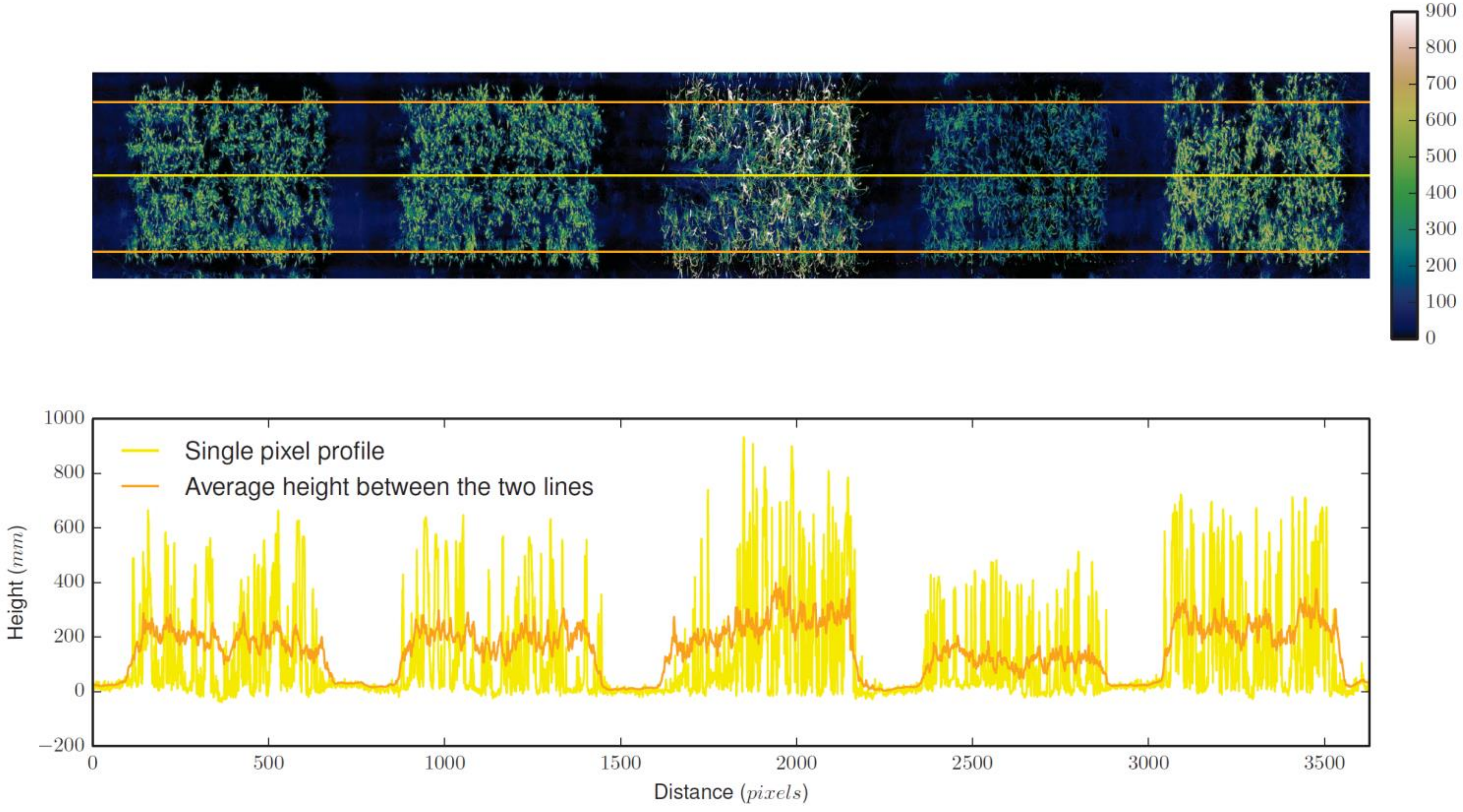
LiDAR

LiDAR – Light Detection and Ranging

the environment is scanned with a pulsed laser beam and the reflection time of the signal from the object back to the detector is measured.

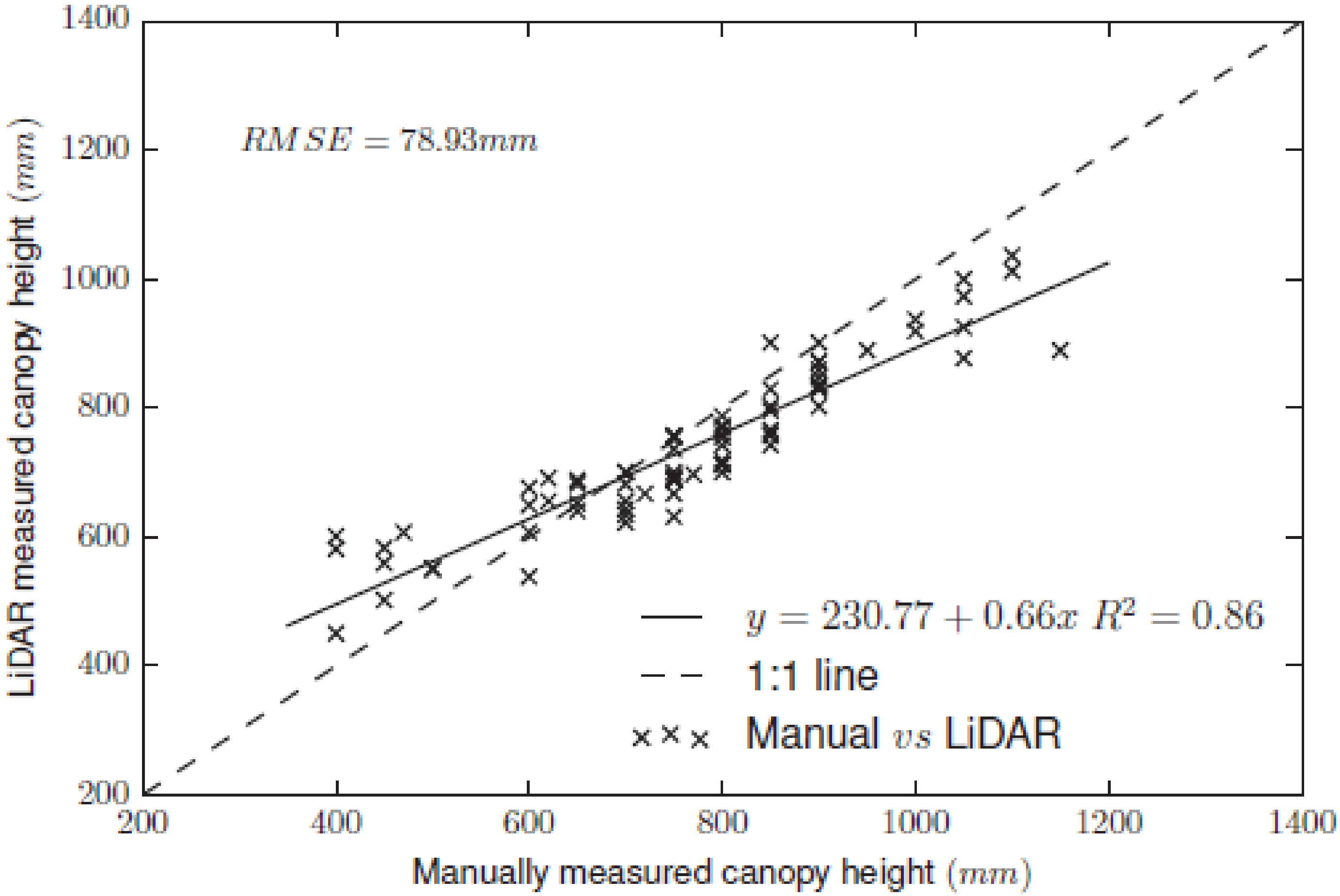
LiDAR

Figure 4. Profile of the LiDAR elevation. The yellow line in the graph represents the profile of the single-pixel width transect across the plots, denoted in yellow in the image; while the orange line in the graph represents the average height of all the pixels between the two orange lines in the image.



Deery *et al.* 2015, *Agronomy*

LiDAR



Deery *et al.* 2015, *Agronomy*

LiDAR

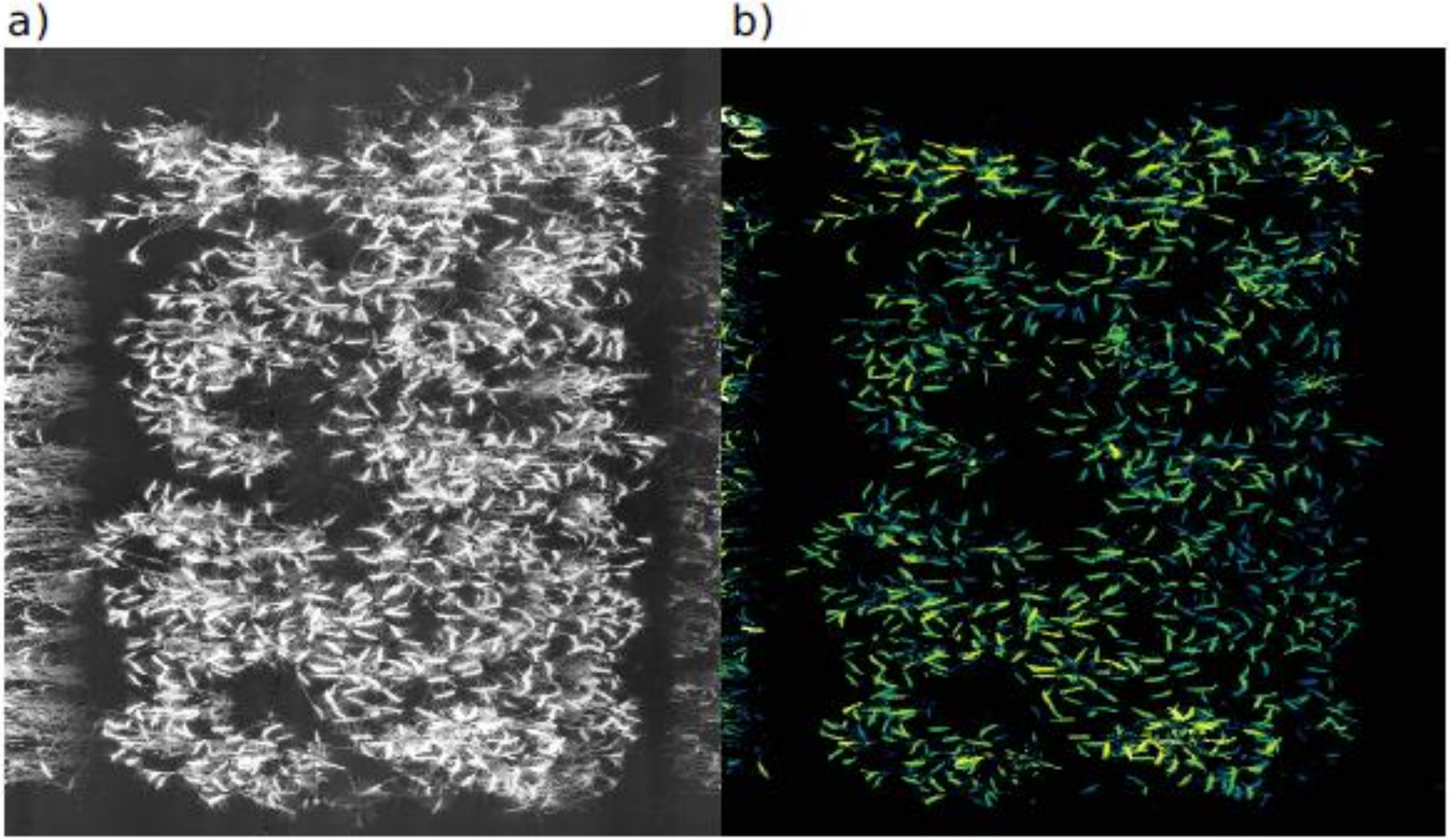


Figure 6. An example of the application of LiDAR for counting spikes in wheat. The LiDAR elevation image (**a**) can be segmented into an image showing only the top fraction of the image, which clearly shows the spikes (**b**). A simple particle count algorithm can be used to count the number of elements per area.

Deery *et al.* 2015, *Agronomy*

RGB images

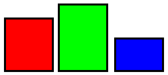
Digital photography

green biomass

Numerical representation of color

There are a number of different systems for representing a given color.

•RGB: Red, Green and Blue



related with color reproduction by computer screens, etc.

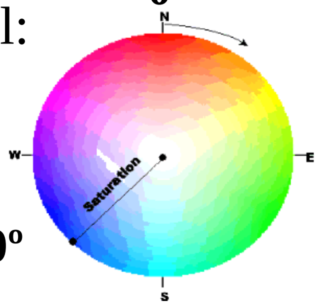
•HIS

Hue, Intensity, Saturation

Practical for image analysis

Hue wheel:

0º



120º

240º

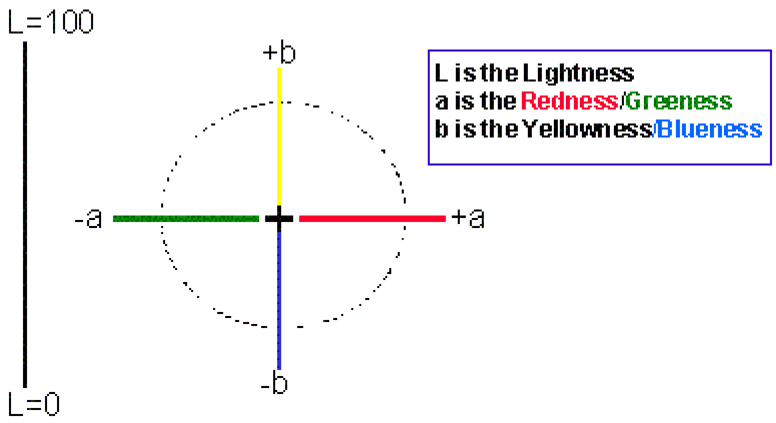
•CIE-lab

~ sensitivity of human visual system

Consistent distance

→ practical for arithmetics

CIE-Lab



RGB image processing: vegetation indices

CIMMYT Maize Scanner for RGB field-based phenotyping (released at http://github.com/george-haddad/CIMMYT)

Calculates a number of RGB based indexes for estimating disease impacts, crop vigor, LAI, biomass at the leaf and canopy scale, including Breedpix (GA and GGA), Triangle Greeness Index (TGI), and Normalized Green Red Difference Index (NGRDI)

Kefauver *et al.*

RGB, Green Area, Greener Green Area

MLN plot score 3.0

Maize Leaf Plot RGB

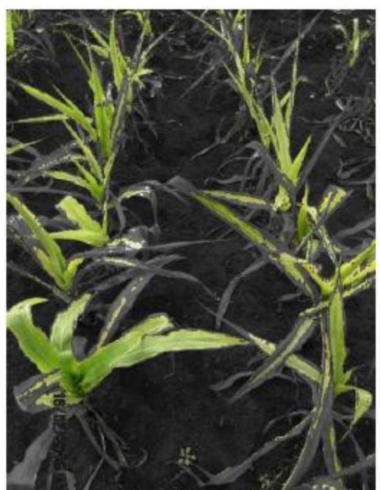
GA (healthy pixels)

GGA (very healthy pixels)

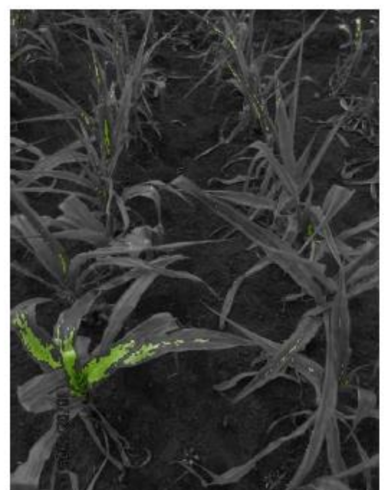
NGRDI (vigor index)

MLN plot score 4.0

Maize Leaf Plot RGB



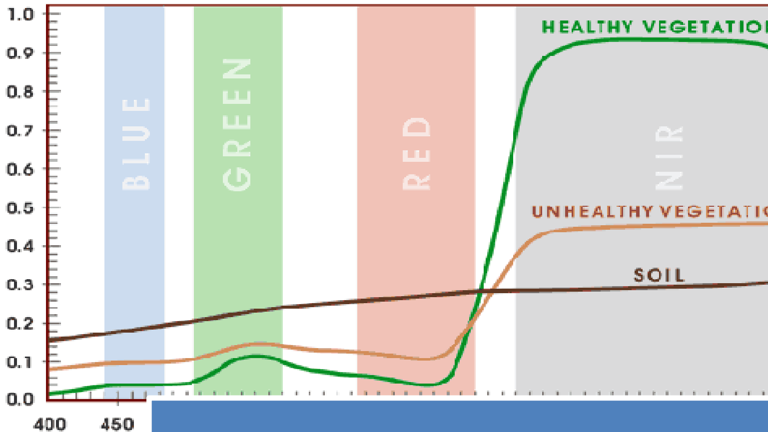
GA (healthy pixels)



GGA (very healthy pixels)

NGRDI (vigor index)

Kefauver *et al.*



RGB indexes have a more limited spectral range

but they have an **excellent spatial resolution**

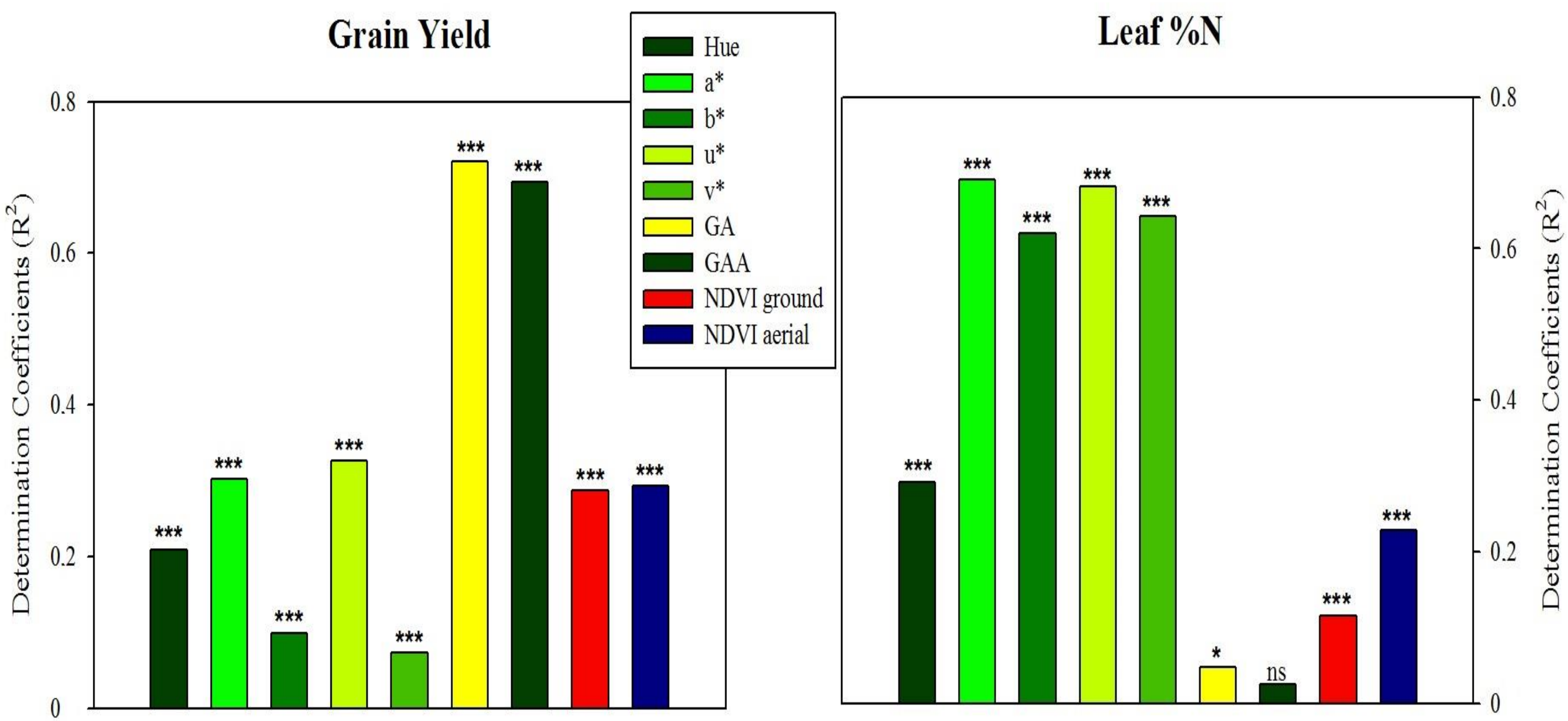
- In most of these studies, RGB indexes outperformed NDVI

- NDVI may be more affected by:
  - Canopy architecture
  - Crop density
  - Spikes and soil

because of their effect on the reflectance at longer wavelengths.

RGB vs Spectral indices

N fertilization treatments in maize



Vegetation Indexes

Computers and Electronics in Agriculture 116 (2015) 20–29

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Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

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Low-cost assessment of wheat resistance to yellow rust through conventional RGB images

B. Zhou, A. Elazab, J. Bort, O. Vergara, M.D. Serret, J.L. Araus *

Unitat de Fisiologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

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The Crop Journal

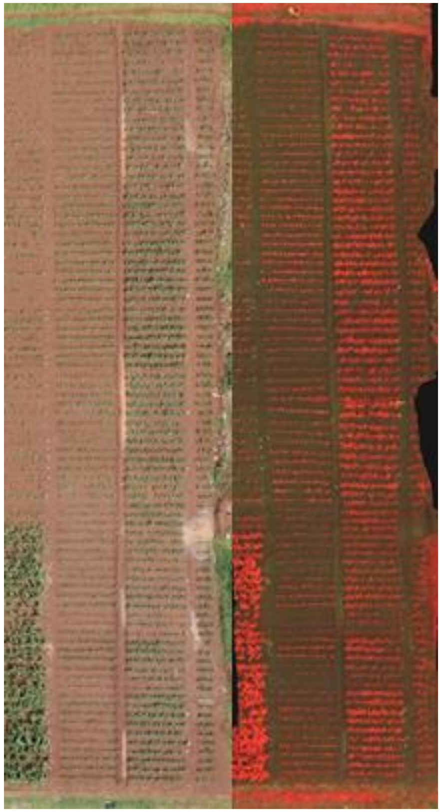
Grain yield losses in yellow-rusted durum wheat estimated using digital and conventional parameters under field conditions

Omar Vergara-Diaz[a], Shawn C. Kefauver[a,], Abdelhalim Elazab[a], Maria Teresa Nieto-Taladriz[b], José Luis Araus[a]*

[a]Unit of Plant Physiology, Department of Plant Biology, Faculty of Biology, University of Barcelona, Diagonal 645, 08028 Barcelona, Spain
[b]National Institute for Agricultural and Food Research and Technology (INIA), Ctra de la Coruña 7.5, 28040, Madrid Spain

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant

Phosphorus deficiency in maize



Pearson correlations (R^2) of the different remote sensing indices against grain yield.

RGB Indices / ground			*RGB Indices / aerial*			*Multispectral Indices*		
Intensity	0.007		*Intensity*	0.384	***	*NDVI.ground*	0.745	***
Hue	0.684	***	*Hue*	0.753	***	*NDVI*	0.677	***
Saturation	0.032		*Saturation*	0.055		*SAVI*	0.677	***
Lightness	0.042		*Lightness*	0.277	***	OSAVI	0.639	***
*a**	0.669	***	*a**	0.779	***	*RDVI*	0.687	***
*b**	0.029		*b**	0.085	*	*EVI*	0.008	
*u**	0.617	***	*u**	0.762	***	*PRI*	0.165	**
*v**	0.189	**	*v**	0.000		*MCARI*	0.204	**
GA	0.771	***	*GA*	0.795	***	TCARI	0.060	
GGA	0.770	***	*GGA*	0.701	***	*ARI2*	0.018	
						CRI2	0.008	
						WBI	0.358	***

Gracia et al. 2017 Front.Plant Sci. (in press)

Wheat – yellow rust

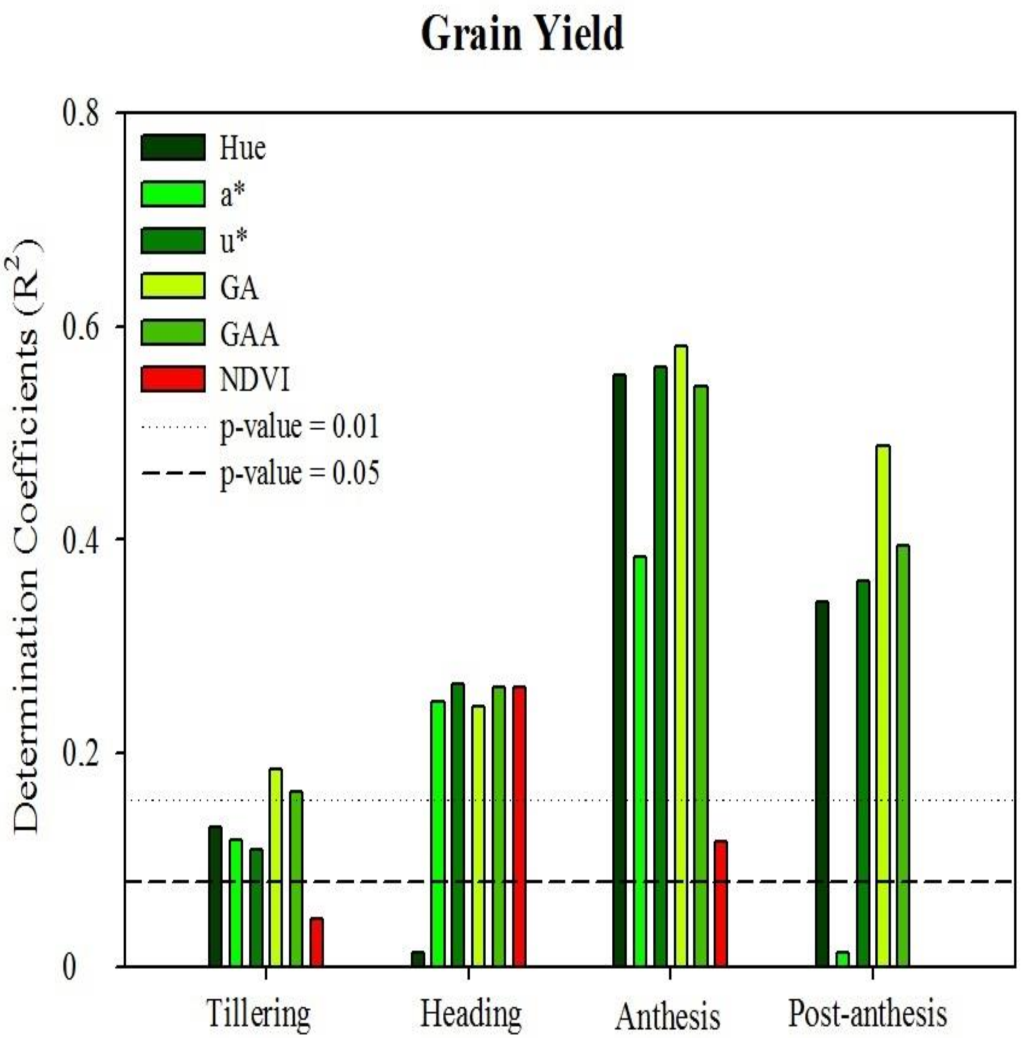


Fig. 1 – Wheat leaves damaged by yellow rust during 2012–2013.

THE CROP JOURNAL 3 (2015) 200–210

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The Crop Journal

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Unitat de Fisiologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain

Other applications of RGB images: harvest index

Kernels

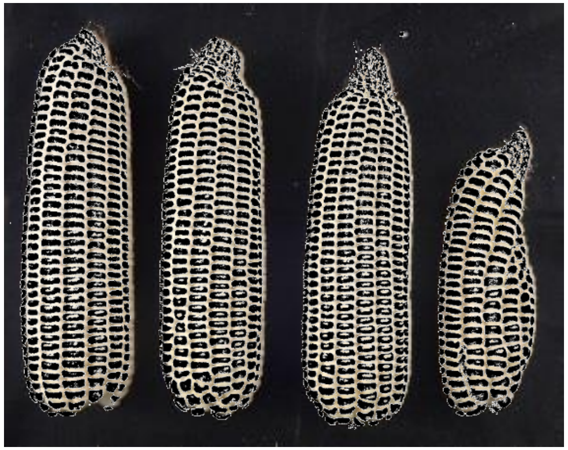
- Size
- Uniformity/abortion rate
- Kernel weight
- Rot index

Ears

- Number
- Rows
- Size

Stress stage

- Flowering stress
- Grain filling stress



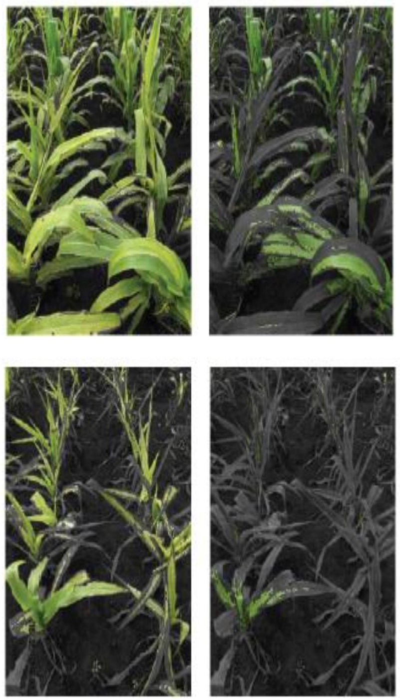
Zaman-Allah *et al.* submitted

Image analysis for diseases

Maize lethal necrosis

Visual score = 3

Visual score = 4

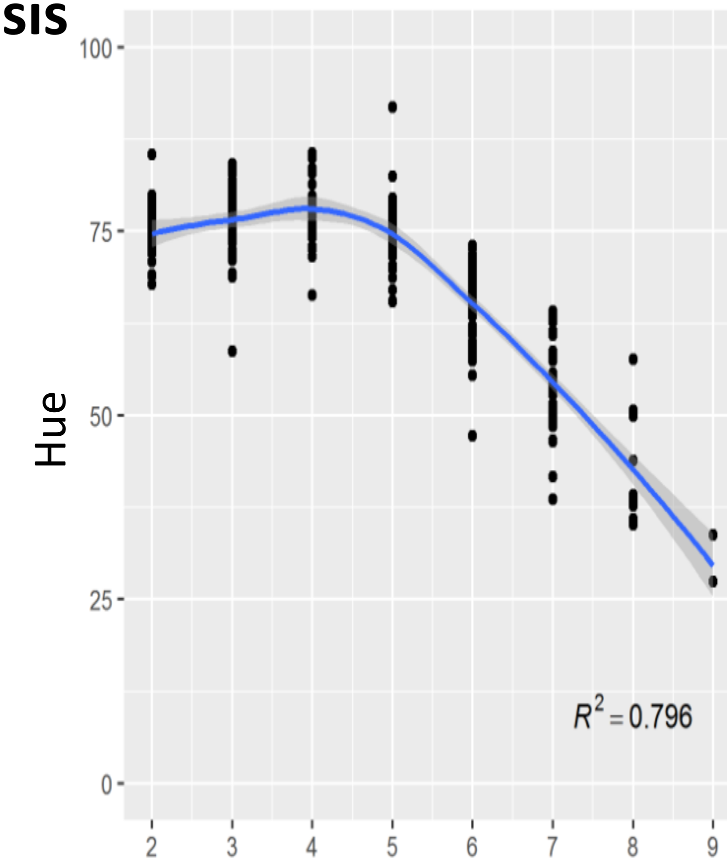


Green Area

(% pixels hue 60-180°)

Greener Area

(% pixels hue 80-180°)

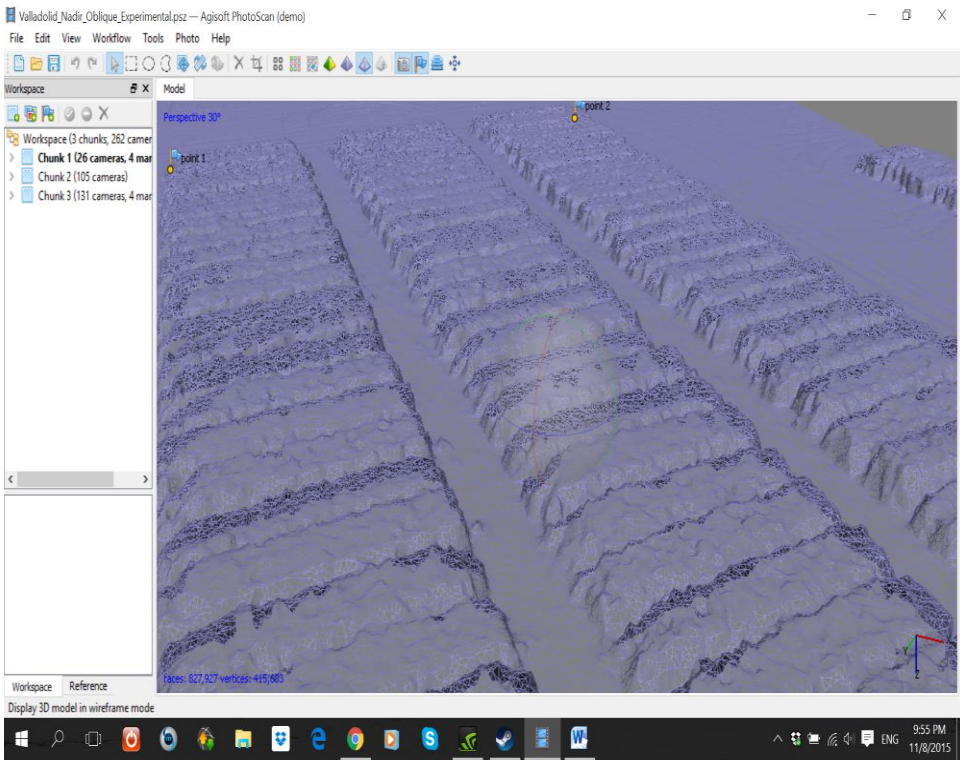


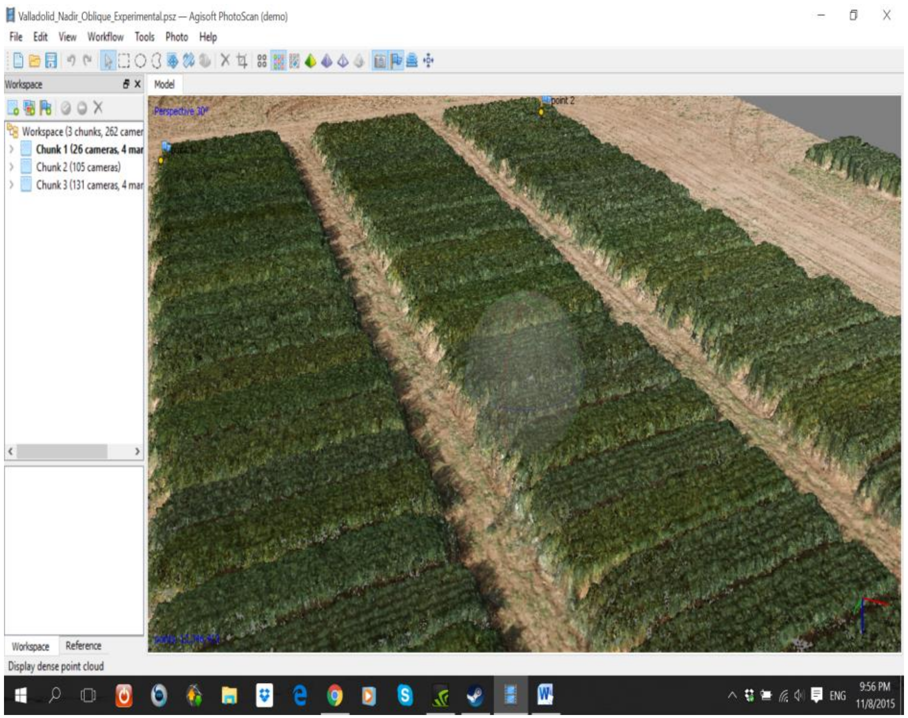
Visual maize lethal necrosis (MLN) score

Kefauver et al. *in preparation*

Other uses RGB images

3D modelling done in Agisoft: the blue 3D mesh (left) and the combination of the 3D model and the color photos creates the color 3D image (right).





S.C. Kefauver

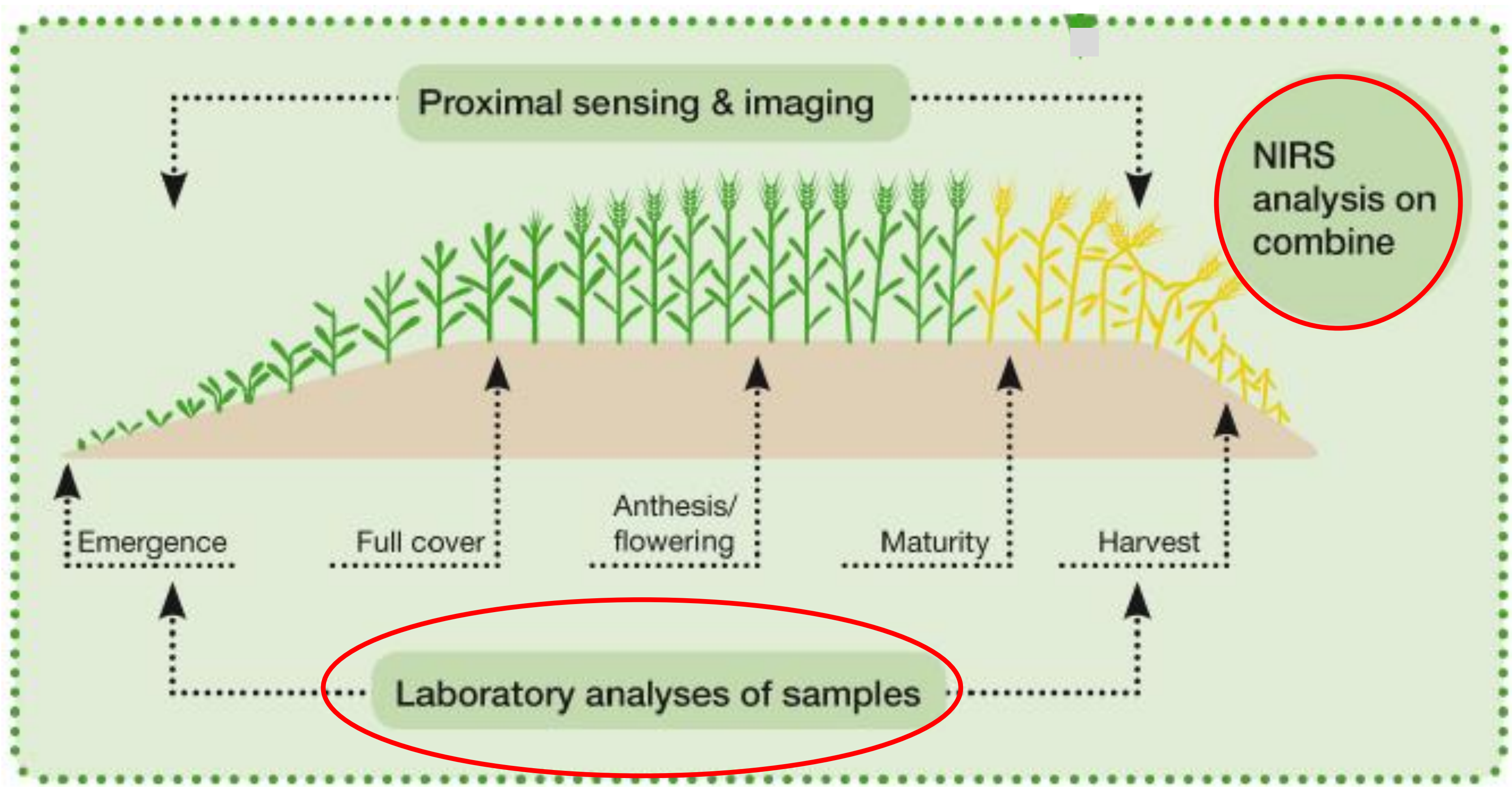
Applications and limitations of sensors

Table 2. Applications and limitations of common sensors mounted on field buggies.

Sensor Type	Applications	Limitations
RGB Cameras	Imaging canopy cover and canopy colour. Colour information can be used for deriving information about chlorophyll concentration through greenness indices. The use of 3D stereo reconstruction from multiple cameras or viewpoints allows the estimation of canopy architecture parameters.	No spectral calibration, only relative measurements. Shadows and changes in ambient light conditions can result in under- or over-exposure and limit automation of image processing.
LiDAR and time of flight sensors	Canopy height and canopy architecture in the case of imaging sensors (e.g., LiDAR). Estimation of LAI, volume and biomass. Reflectance from the laser can be used for retrieving spectral information (reflectance in that wavelength).	Integration/synchronization with GPS and wheel encoder position systems is required for georeferencing.
Spectral sensors	Biochemical composition of the leaf/canopy. Pigment concentration, water content, indirect measurement of biotic/abiotic stress. Canopy architecture/LAI with NDVI.	Sensor calibration required. Changes in ambient light conditions influence signal and necessitate frequent white reference calibration. Canopy structure and camera/sun geometries influence signal. Data management is challenging.
Fluorescence	Photosynthetic status, indirect measurement of biotic/abiotic stress.	Difficult to measure in the field at the canopy scale, because of the small signal-to-noise ratio, though laser-induced fluorescence transients (LIFT) can extend the range available, while solar-induced fluorescence can be used remotely.
Thermal sensors	Stomatal conductance. Water stress induced by biotic or abiotic factors.	Changes in ambient conditions lead to changes in canopy temperature, making a comparison through time difficult, necessitating the use of references. Difficult to separate soil temperature from plant temperature in sparse canopies, limiting the automation of image processing. Sensor calibration and atmospheric correction are often required.
Other sensors: electromagnetic induction (EMI), ground penetrating radar (GPR) and electrical resistance tomography (ERT)	Mapping of soil physical properties, such as water content, electric conductivity or root mapping.	Data interpretation is challenging, as heterogeneous soil properties can strongly influence the signal.

Deery *et al.* 2014 *Agronomy*

Different categories of traits

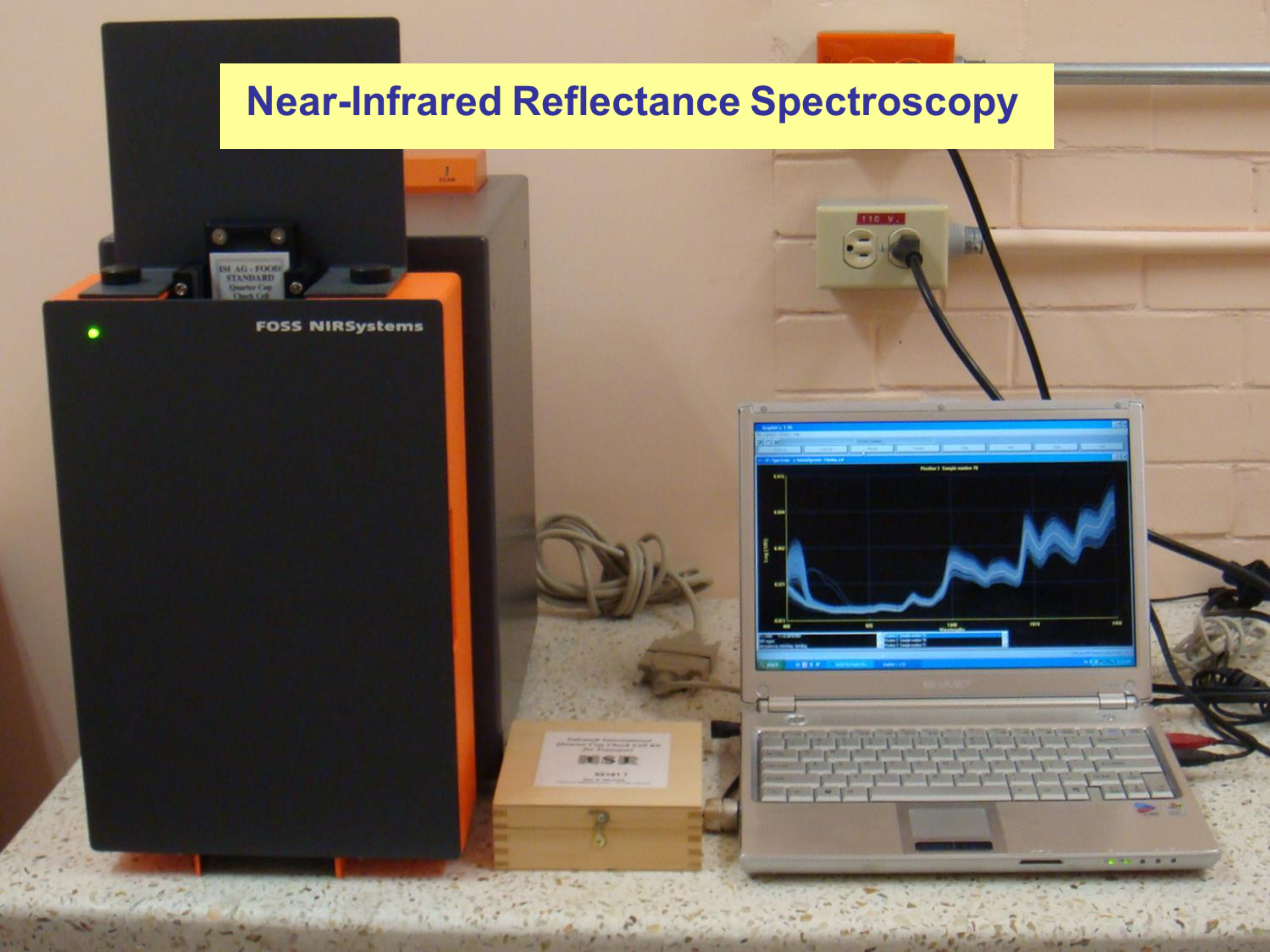


Stable isotopes: $\Delta^{13}C$ & Yield

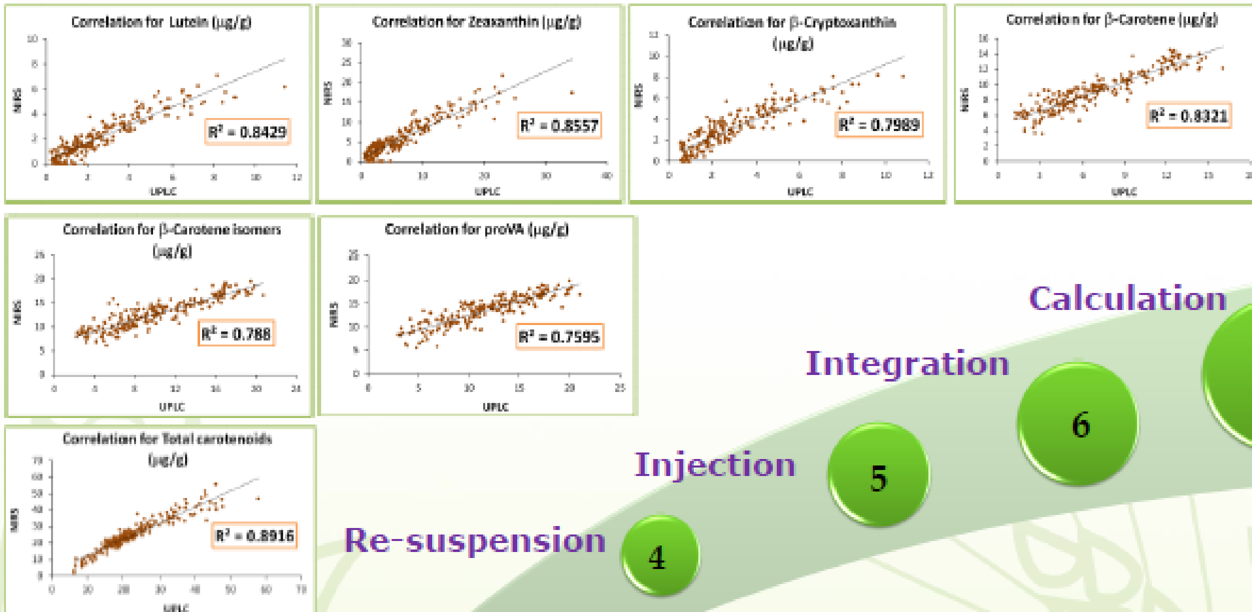
'Drysdale (2002) and Rees (2003) are drought tolerant wheat varieties bred by CSIRO scientists using innovative gene selection criteria. The DELTA technique gives plant breeders the ability to breed varieties of wheat that more efficiently exchange atmospheric carbon dioxide for water during photosynthesis'

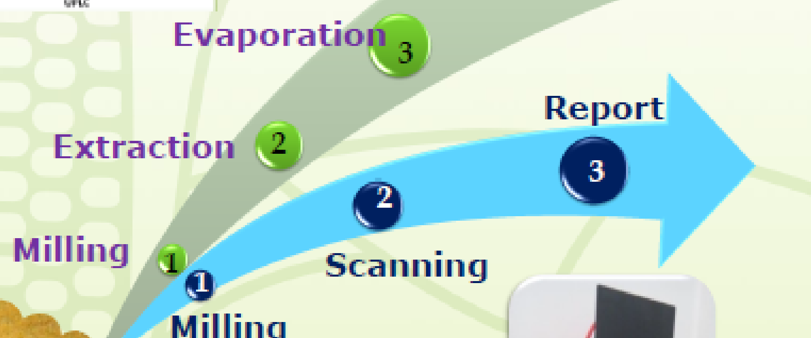
They were selected for low $\Delta^{13}C$ increased WUE as crop mostly grows on storage water which exhausted through the growing season

Near-Infrared Reflectance Spectroscopy



UPLC and NIR for carotenoids in maize





UPLC

NIRS

Factor	UPLC	NIRS
Accuracy	Quant. Qual.	Quant.
Time of analysis	~3 h	~ 4 min
Cost/sample	37.10 US	2.62 US
Risk		
Waste generation		
HR	3 people	1 person

Rosales et al., forthcoming

1966 - 2016 CIMMYT

CIMMYT

Comparative of cost and time

Technique	IRMS		EA	AACC Method	NIRS-prediction			
Parameter	$\delta^{13}C$	$\delta^{18}O$	N content	Ash content	$\delta^{13}C^{*}$	$\delta^{18}O$	Ash	N
Cost per sample	10€	20€	3€	1.5€	0.5€			
Time	<10 min	<10 min	<10 min	≈24 h	≈3 min			
Equipment	EA-IRMS		EA	Muffle furnace	NIR spectrometer			

*previously reported by Clark *et al*. 1995; Ferrio *et al*. 2001; Kleinebecker *et al*. 2009

Outline

Phenotyping

- A bottleneck for breeding
- Current challenges
- Identifying the traits
- Selecting the tools for field phenotyping
- Effective and expensive are not synonyms
- **Platforms**
- More than traits, tools and platforms

Fixed field phenotyping platforms

Field scanalyzer at Rothamsted Research, Harpenden

The world's largest robotic field scanner (white steel box) is mounted on a 30-ton steel gantry moving along 200-meter steel rails over 1.5 acres of energy sorghum at the Maricopa Agricultural Center. (Photo: Susan McGinley)

The ETH field phenotyping platform FIP: a cable-suspended multi-sensor system

Cranes - towers

The canopy: structure-function relationships

flexible system – cherry picker

SLR-cameras on a sliding bar
+ hyperspectral imaging

Rascher et al. 2011, FPB 38: 968-983

Laser / LIDAR – detailed maps of the outer canopy

3D Stereo imaging: structural features – e.g. leaf orientation

Hyperspectral imaging:
- NDVI - chlorophyll
- PRI - photosynthestic efficiency - influenced by chlorophyll and canopy structure

Stereo imaging – quantitative description of relevant canopy elements

Phenomobiles

	Description
1	Frame with 1.5 m ground clearance
2	Wheel encoders (~1-mm accuracy)
3	Real time kinematic GPS (~2-cm accuracy)
4	Height adjustable boom (max 3 m)
5	Removable light bank
6	Three LiDAR sensors
7	Four RGB stereo cameras
8	Spectrometer/ hyperspectral camera
9	Infra-red thermometers/ infra-red thermal camera
10	Generator and electronics
11	Two wheel drive hydraulic drive system

Deery *et al.* 2014 *Agronomy*

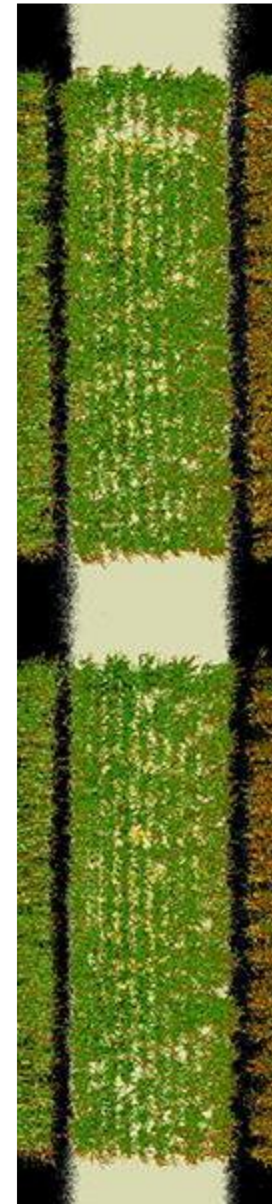
Rebetzke *et al.* 2013 *Funct. Plant Biol.*

PHENOMOBILE LITE

Advanced high throughput field phenotyping buggy

Typical Applications

The Phenomobile Lite has been successfully validated to be a surrogate for the non-destructive field phenotyping of both wheat and rice yielding estimates of canopy height, fractional ground cover, greenness vertical distribution, leaf area, plant counts, visual assessments, and with optional NDVI GreenSeeker, canopy density and greenness.



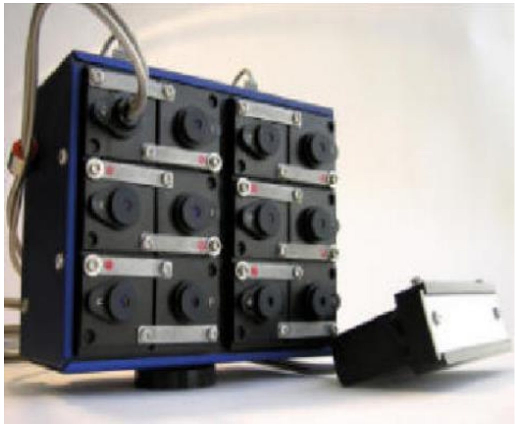
Phenocart

White and Conley 2013 Crop Sci. 53: 1646-1649

Aerial platforms

M. Reynolds, Ciudad Obregon, Mexico

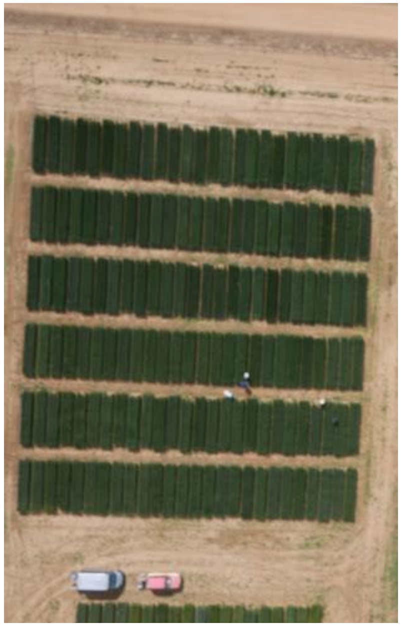
University of Barcelona Current HTPP



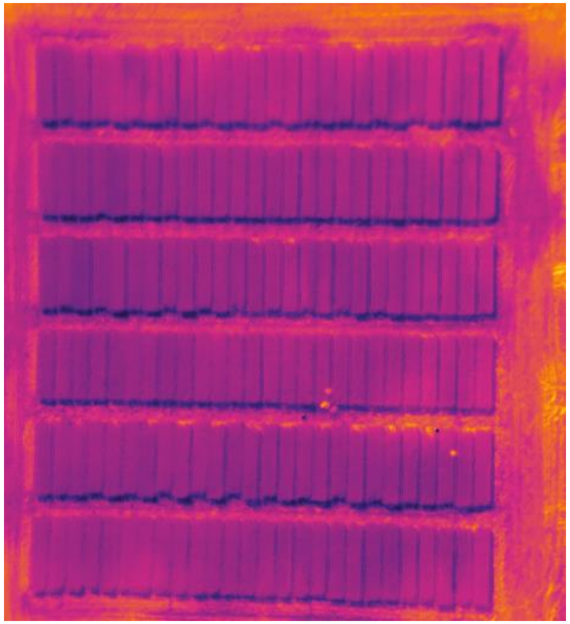
Multispectral Tetracam 11+ILS

TEAX ThermalCapture FLIR
Tau 640 Camera

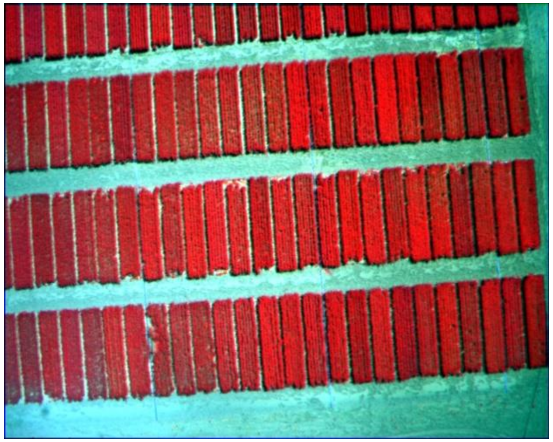
Lumix GX7 16 MP RGB camera



RGB

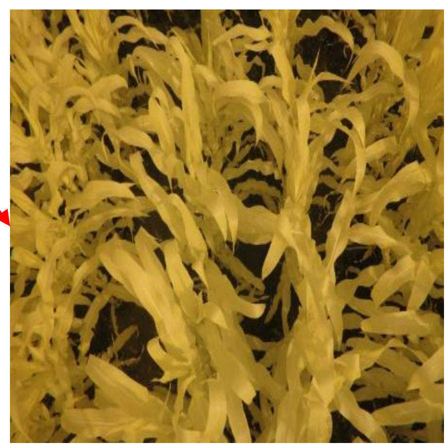


Thermal



Multispectral

In the case of projects where UAVs are prohibited…



RGB camera
(Sony QX1)

- Images taken with a *phenopole*.
- Zenithal.
- More canopy area evaluated
- Soil (and sky) background effect reduced.

NDVI modified camera
(Canon S100)

Kefauver et al. *in preparation*

Naivasha, Kenya

Table 1. Comparison of hand-held, cart, and tractor-based proximal sensing.

Characteristic	Hand-held	Cart	Tractor
Initial cost	Low	Low	High
Cost of operation	Moderate	Moderate	Moderate
Ease of maneuvering within field trials	High	Moderate	Low
Ease of maintaining a precise sensor height	Moderate	High	High
Ease of simultaneously deploying multiple sensors	Low	High	High
Ease of simultaneously deploying multiple sets of sensors over different rows	Low	Moderate	High
Ease of stop-and-go operation	High	High	Moderate
Maximum clearance	High	Moderate	Moderate
Risk of person or vehicle interfering with reflectance or thermometric sensor readings	Low	Low	Moderate
Risk of soil compaction	Low	Low	Moderate
Risk of damage to plants in a closed canopy	Low	Moderate	High
Ease of adjustment for different row spacings	High	Medium	Low
Ease of transport	High	Medium	Low

White and Conley 2013 Crop Sci. 53

2016 Nano/Microsatellite Applications and Associated Examples

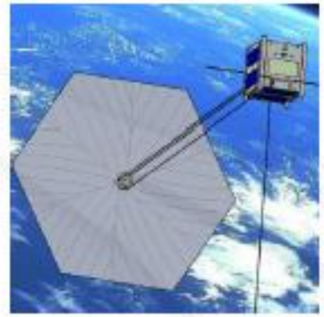
Credit: http://space.skyrocket.de

Communications

ITF 2

Mass: 1.3 kg

Launched: 12/2016



Credit: JAXA

Technology

Waseda-SAT 3

Mass: 1.3 kg

Launched: 12/2016

Credit: Satellogic

Earth Observation

NuSat 1 (Aleph-1)

Mass: 37 kg

Launched: 5/2016

Credit: Earth Observation Portal

Scientific

RAVAN

Mass: 5 kg

Launched: 11/2016

Credit: http://space.skyrocket.de

Technology

CELTEE 1

Mass: 1.3 kg

Launched: 11/2016

Credit: Spire

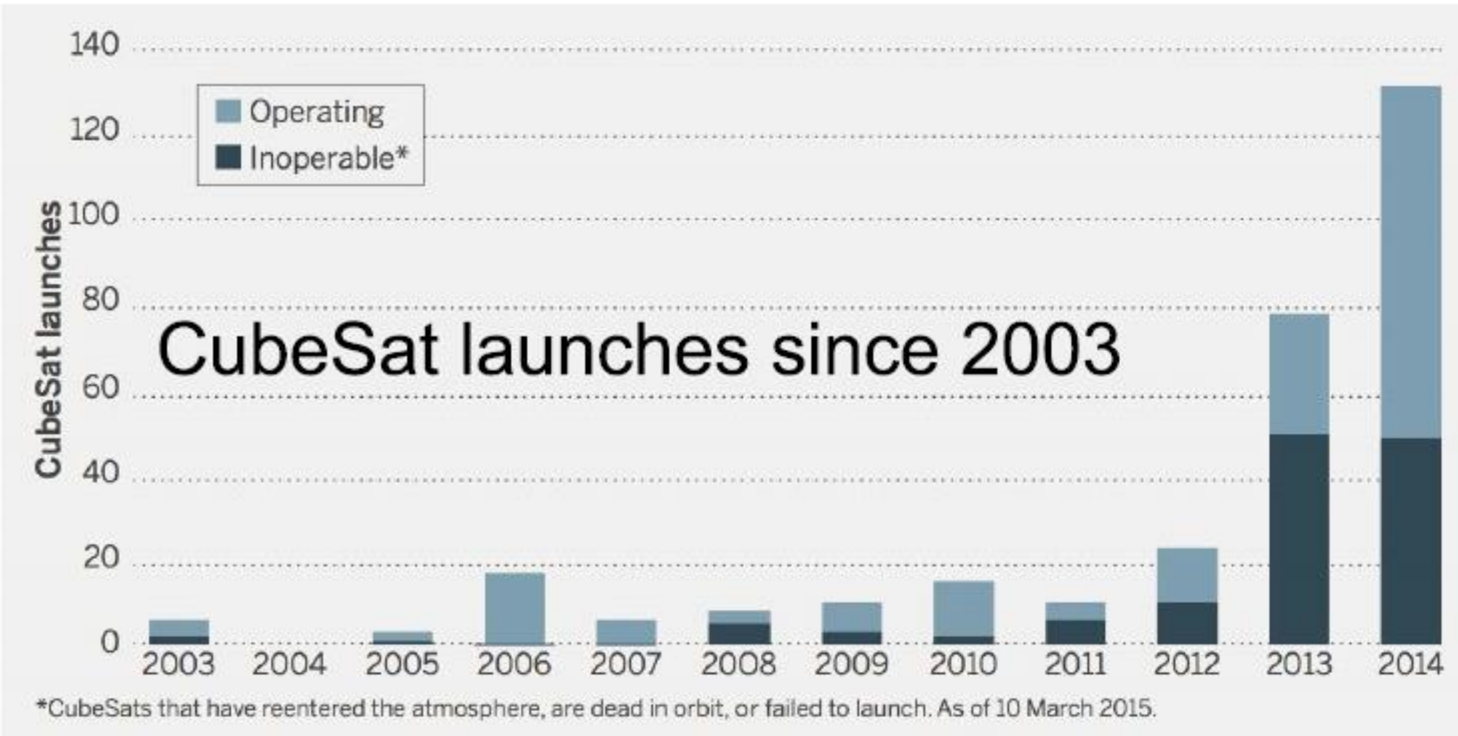
Remote Sensing

Lemur-2

Mass: 5 kg

Launched: 5/2016

A Golden Age for smallholder remote sensing…



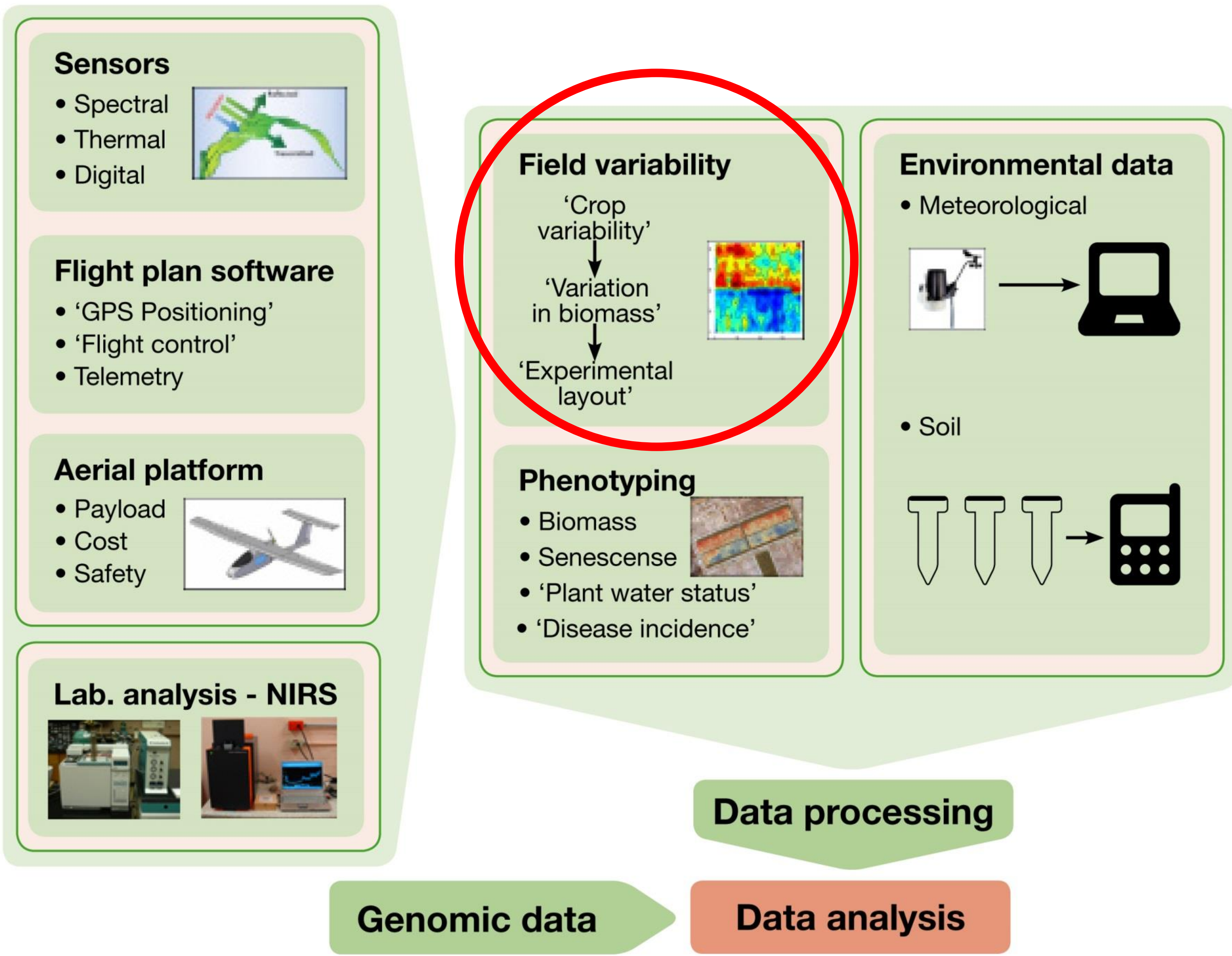
Hand, *Science* News, 2015

Lobell, CIMMYT 50

Outline

Phenotyping

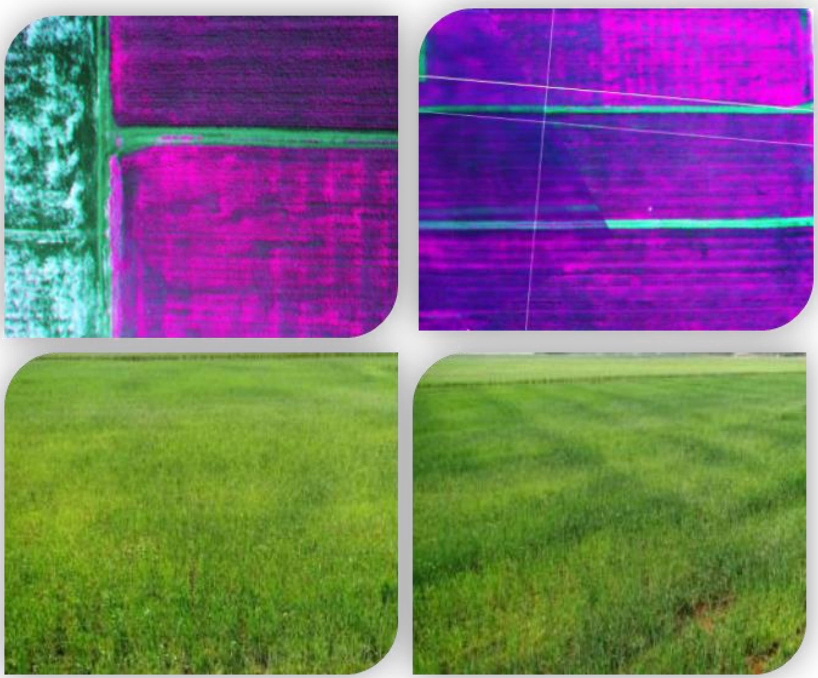
- A bottleneck for breeding
- Current challenges
- Identifying the traits
- Selecting the tools for field phenotyping
- Effective and expensive are not synonyms
- Platforms
- **More than traits, tools and platforms**


Sensors
• Spectral
• Thermal
• Digital
Flight plan software
• 'GPS Positioning'
• 'Flight control'
• Telemetry
Aerial platform
• Payload
• Cost
• Safety
Lab. analysis - NIRS
Field variability
'Crop variability'
'Variation in biomass'
'Experimental layout'
Phenotyping
• Biomass
• Senescense
• 'Plant water status'
• 'Disease incidence'
Environmental data
• Meteorological
• Soil
Data processing
Genomic data
Data analysis

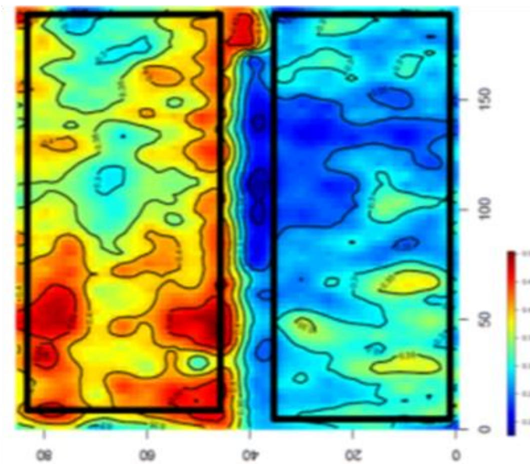
Environmental variability

Within-site variability

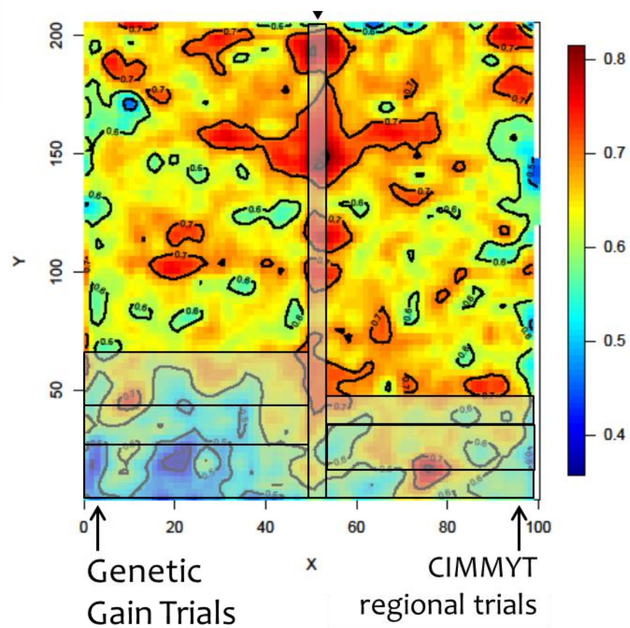
Measuring / reducing spatial variability



Cairns and Zaman-Allah



Masuka et al. 2012



Das and Crossa

Reducing field variability: managed growth conditions

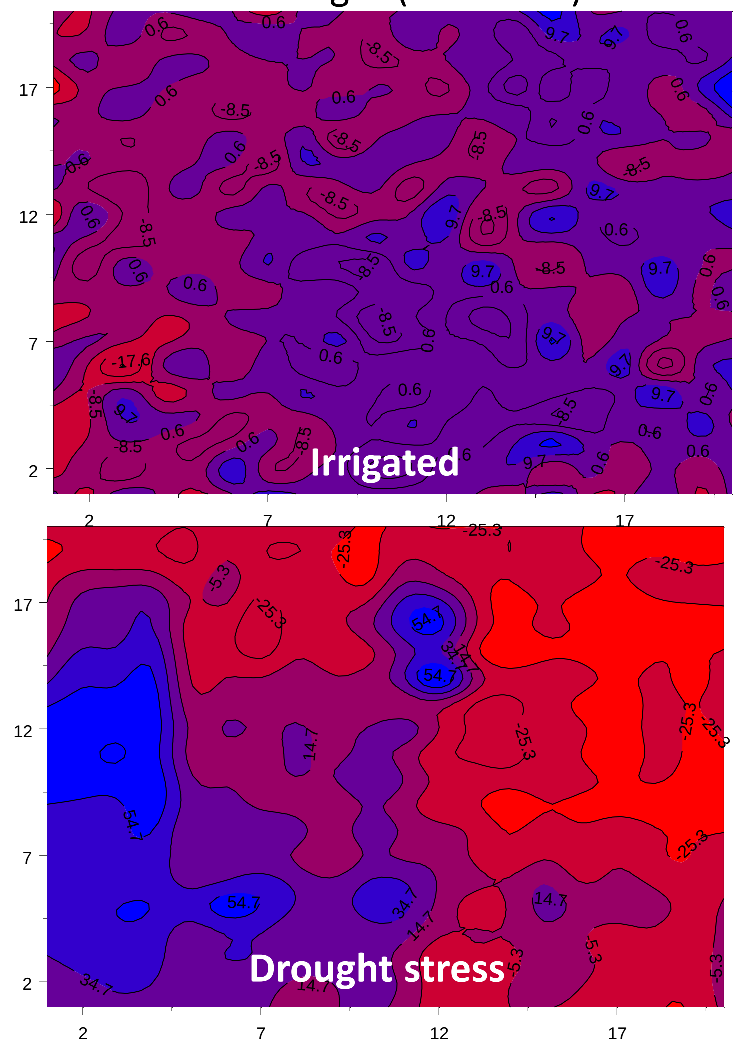
Variance components†	Well-watered	Drought stress	Combined drought and heat stress
σ^2_g	0.35	0.12	0.07
$\sigma^2_{g\times e}$	0.24	0.36	0.12
σ^2_e	0.48	0.39	0.18
No. of locations	7	7	3
H	0.84	0.64	0.50

Cairns *et al.* 2013 *Crop Sci.*

Test environment	Variance components‡		
	σ^2_g	σ^2_{ge}	σ^2_ε
Early maturity group			
Optimal	28.02 ± 11.14	24.17 ± 8.24	47.81 ± 13.95
Managed drought	14.39 ± 9.30	14.58 ± 4.17	71.04 ± 8.08
Random abiotic stress	10.29 ± 8.32	23.37 ± 11.76	66.34 ± 14.75
Low N	19.01 ± 10.66	23.86 ± 11.30	57.13 ± 14.18
Late maturity group			
Optimal	22.26 ± 4.50	22.41 ± 7.11	55.34 ± 7.85
Managed drought	17.57 ± 9.43	15.72 ± 8.33	66.70 ± 13.52
Random abiotic stress	10.28 ± 7.28	18.25 ± 6.39	71.47 ± 11.23
Low N	15.69 ± 6.95	15.35 ± 4.77	68.95 ± 8.84

Weber *et al.* 2012 *Crop. Sci.*

Plant Height (Residuals)



Burgueño and Wilcox

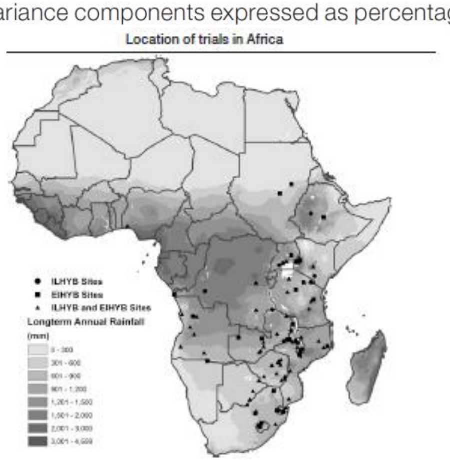
Managed-stress screening

Table 2. Mean and standard deviation of maize grain yield, variance components, and broad-sense heritability (*H*) of grain yield under optimal, managed drought, random abiotic stress, and low-N conditions from 2001 to 2009 as well as predictions of *H* assuming testing in five trials (in italics).

Test environment	N_{Gen}†	N_C†	N_{Env}†	Grain yield (t ha^{-1})	Variance components‡ σ^2_g	σ^2_{ge}	σ^2_ε	*H* (whole set)	Predicted *H* (N_{Env} = 5)
Early maturity group									
Optimal	219	17	201 (217)	5.53 ± 0.61	28.02 ± 11.14	24.17 ± 8.24	47.81 ± 13.95	0.92 ± 0.04	0.85 ± 0.07
Managed drought	210	5	17 (22)	2.29 ± 0.96	14.39 ± 9.30	14.58 ± 4.17	71.04 ± 8.08	0.44 ± 0.21	0.52 ± 0.21
Random abiotic stress	204	13	74 (88)	1.85 ± 0.22	10.29 ± 8.32	23.37 ± 11.76	66.34 ± 14.75	0.55 ± 0.22	0.49 ± 0.22
Low N	219	6	44 (49)	2.04 ± 0.59	19.01 ± 10.66	23.86 ± 11.30	57.13 ± 14.18	0.63 ± 0.21	0.63 ± 0.20
Late maturity group									
Optimal	229	14	175 (187)	6.26 ± 0.39	22.26 ± 4.50	22.41 ± 7.11	55.34 ± 7.85	0.91 ± 0.03	0.68 ± 0.06
Managed drought	216	5	22 (24)	2.11 ± 0.35	17.57 ± 9.43	15.72 ± 8.33	66.70 ± 13.52	0.56 ± 0.19	0.49 ± 0.16
Random abiotic stress	229	10	63 (80)	1.73 ± 0.42	10.28 ± 7.28	18.25 ± 6.39	71.47 ± 11.23	0.61 ± 0.19	0.38 ± 0.16
Low N	220	6	34 (37)	1.82 ± 0.53	15.69 ± 6.95	15.35 ± 4.77	68.95 ± 8.84	0.62 ± 0.14	0.49 ± 0.12

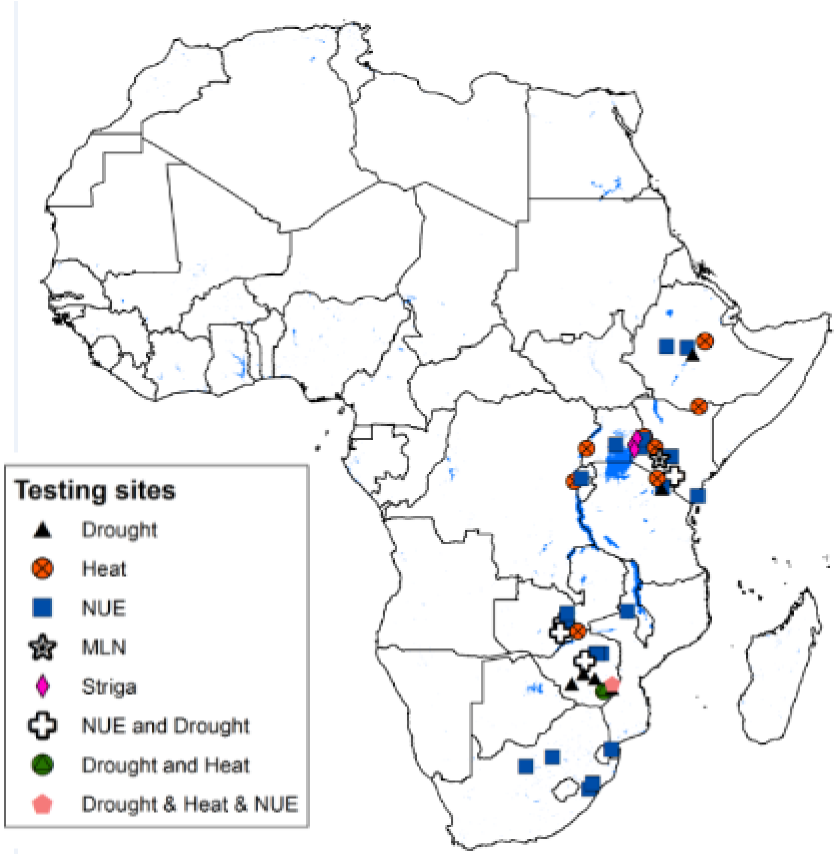
†Total number of genotypes (N_{Gen}), countries (N_C), and environments constituting all location–trial combinations (N_{Env}). The total number of environments excluding and including (in parenthesis) those with repeatability (w^2) < 0.15 is given.

‡Variance components expressed as percentage of the phenotypic variance including the genotype (σ^2_g), the genotype × environment (σ^2_{ge}), and the residual variance (σ^2_ε).

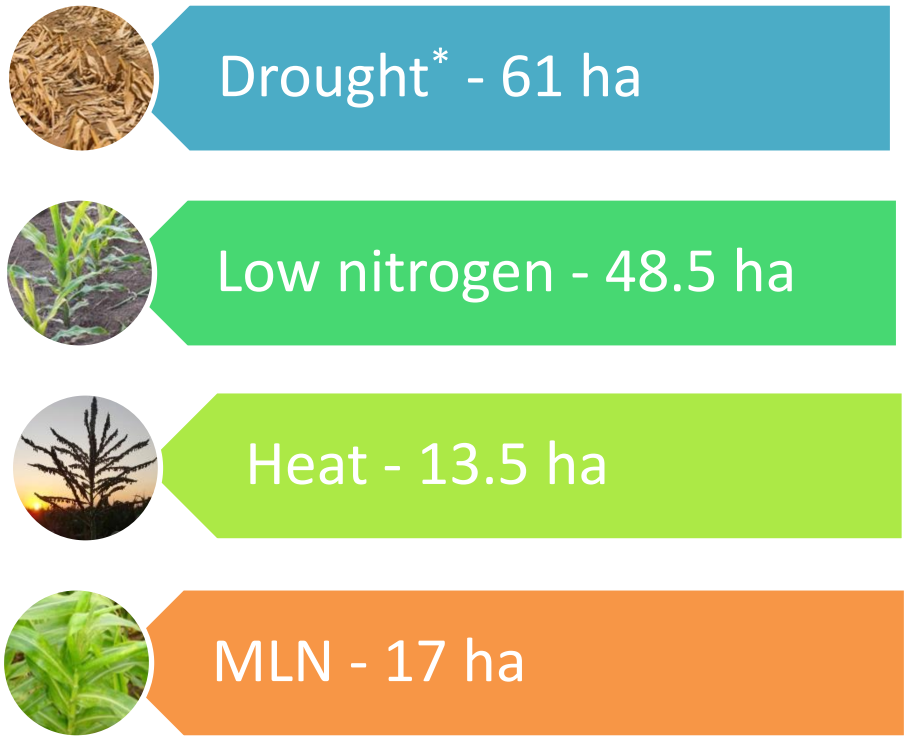


Weber *et al.* 2012 *Crop Sci.*

Large testing network

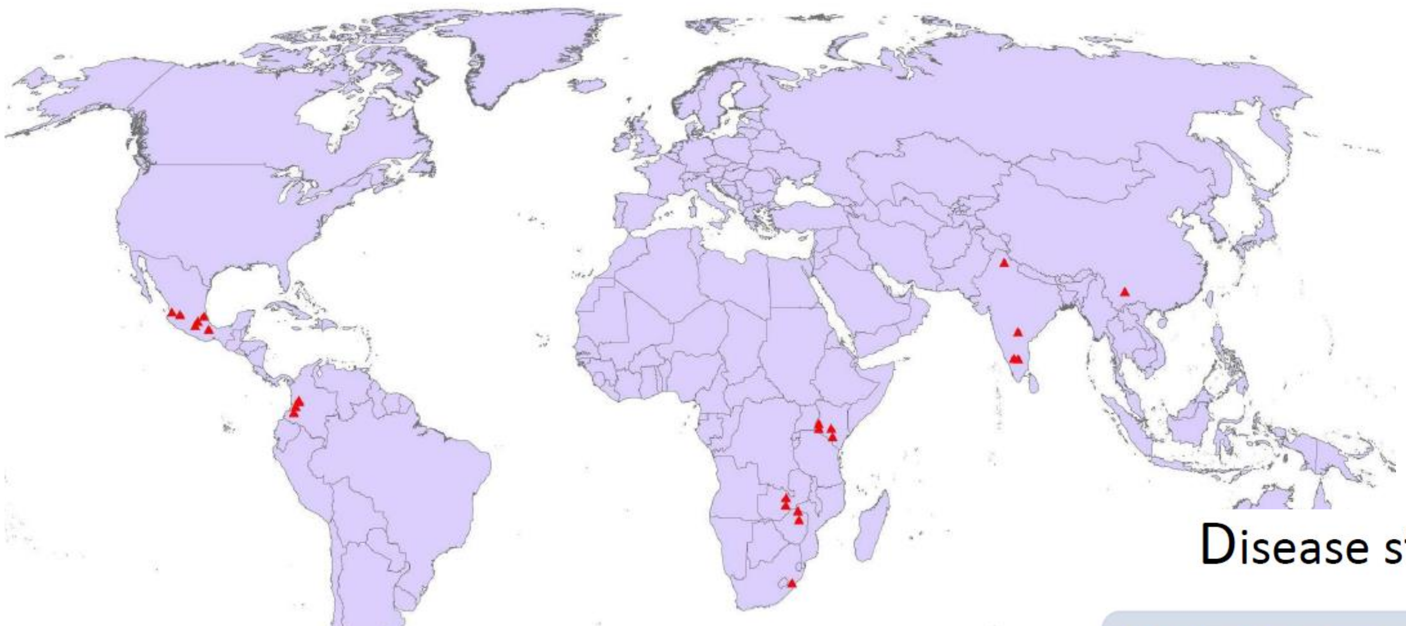


updated from Prasanna et al. 2013



*Including CFT sites

Disease phenotyping sites



Disease stress imposition

Natural infestation

Hotspot

Artificial infestation

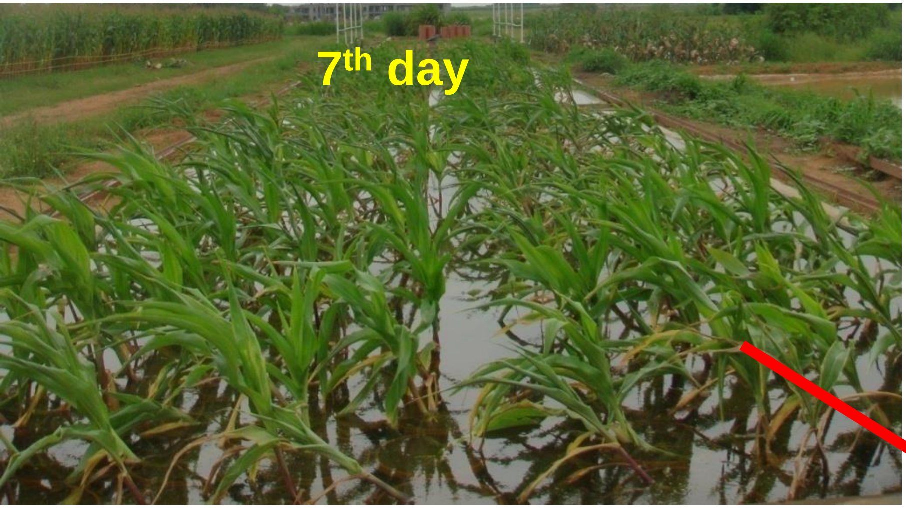
Inoculation

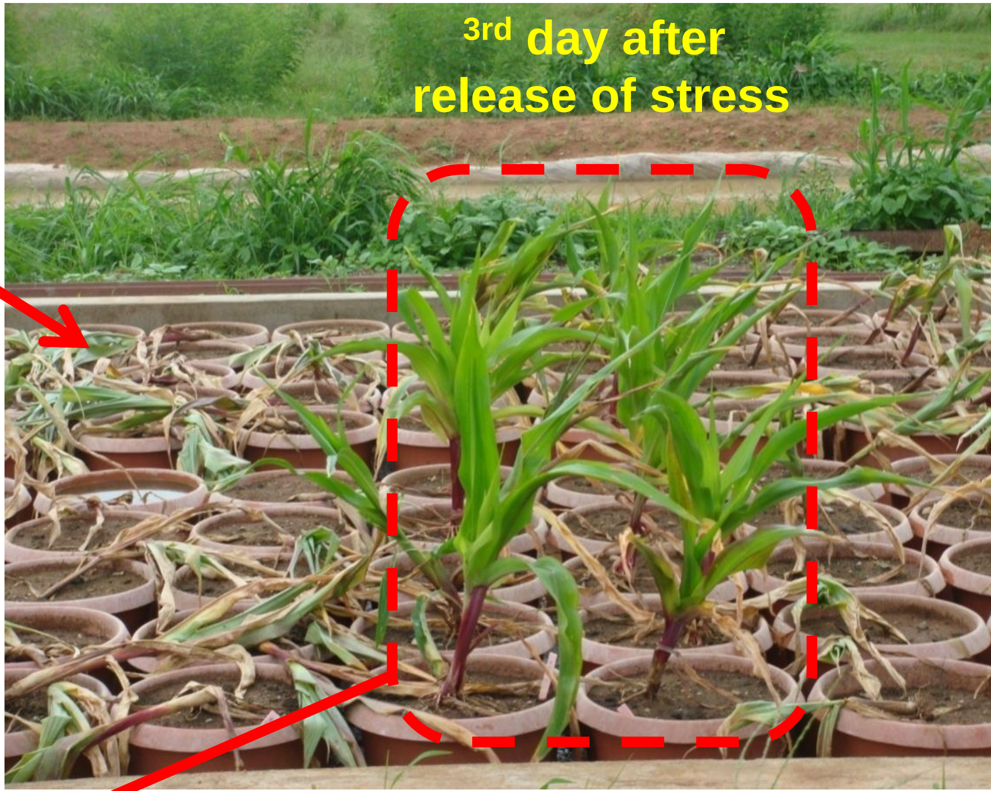
Facilitated infestation

Misting

Adapted from J. Cairns

Water-logging at vegetative growth stage





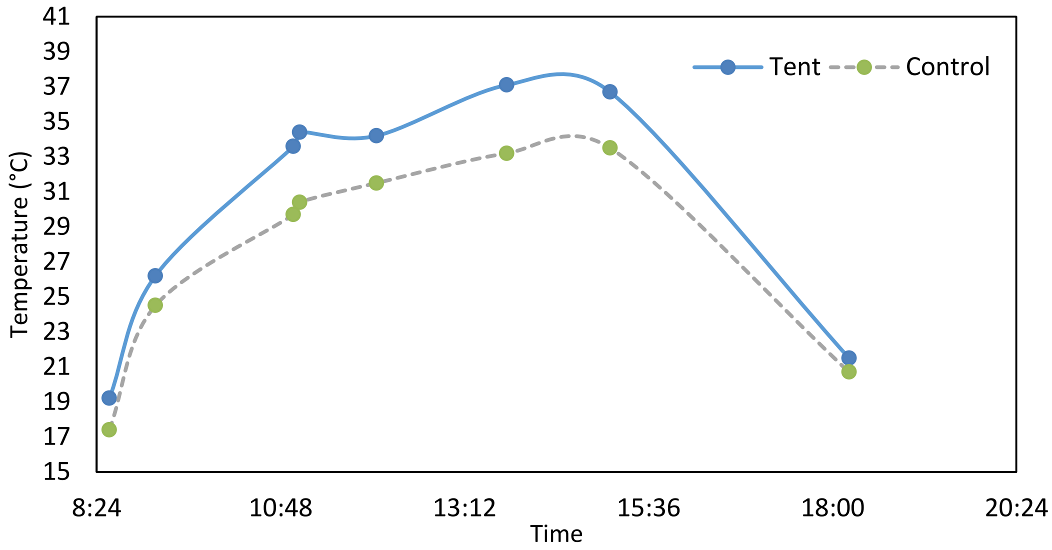
Adapted from Zaidi

Root phenotyping

- ***Structural traits:*** ***root depth, length , volume, root-length density, dry weight***
- ***Functional traits:*** ***water use during stress (WU) & Transpiration efficiency (TE)***

Adapted from Zaidi

Heat stress



a)2-4 days heading **b) 2 days anthesis** **c) 2-4 days A+10**

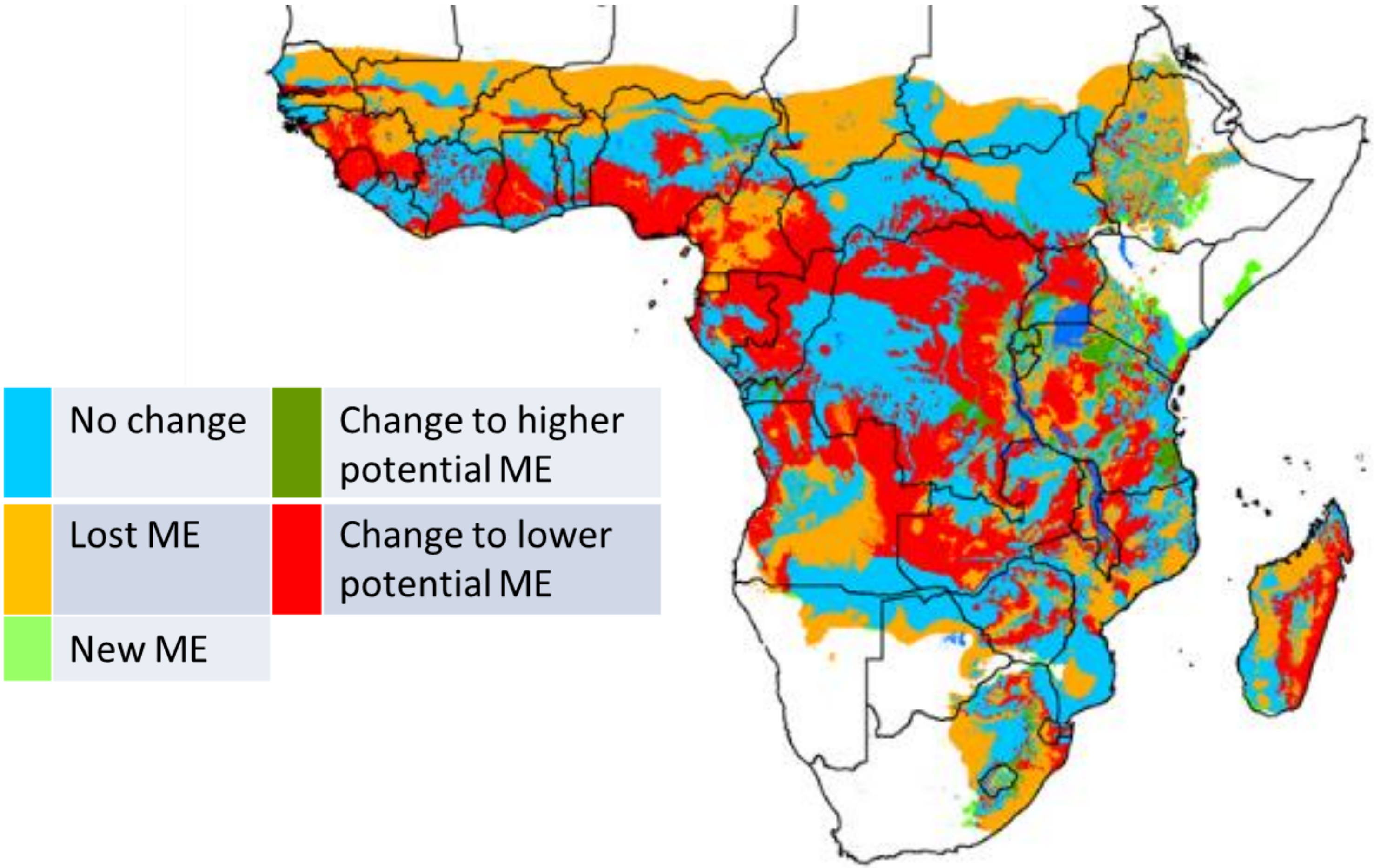
Heat stress

Normal sowing

Late sowing (heat stress)

CENEB CIMMYT, Obregón, México 2015-2016

Aligning breeding programs to future environments



Sonder et al. *submitted*

Harmonized phenotyping protocols for stresses

PHENOTYPING FOR ABIOTIC STRESS TOLERANCE IN MAIZE:

DROUGHT STRESS

M. Zaman-Allah, P.H. Zaidi, S. Trachsel, J.E. Cairns, M.T. Vinayan and K. Seetharam

CIMMYT

PHENOTYPING FOR ABIOTIC STRESS TOLERANCE IN MAIZE:

HEAT STRESS

P.H. Zaidi, M. Zaman-Allah, S. Trachsel, K. Seetharam, J.E. Cairns and M.T. Vinayan

CIMMYT

PHENOTYPING FOR ABIOTIC STRESS TOLERANCE IN MAIZE:

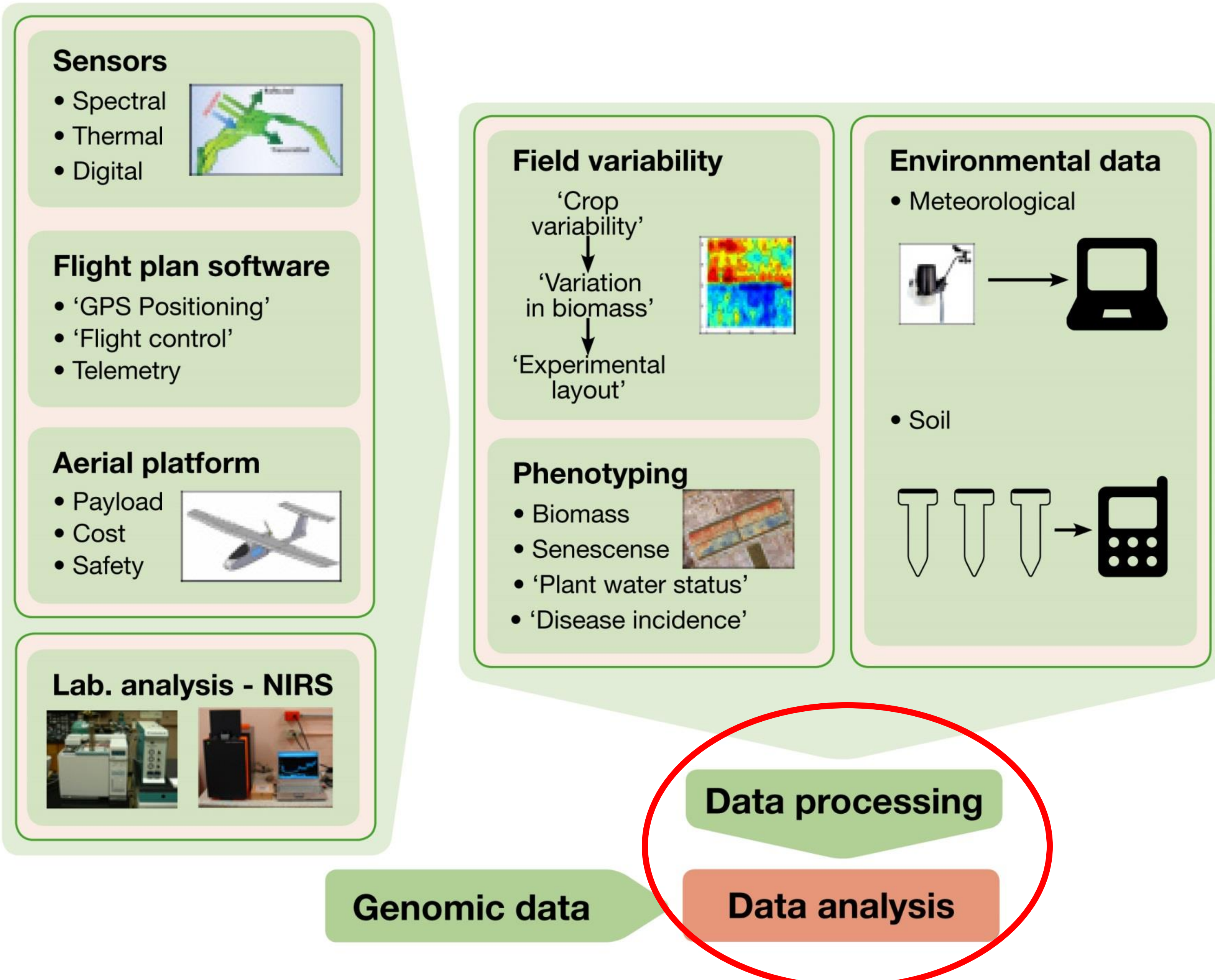
WATERLOGGING STRESS

P.H. Zaidi, M.T. Vinayan and K. Seetharam
CIMMYT Asia Maize Program, Hyderabad, India

CIMMYT

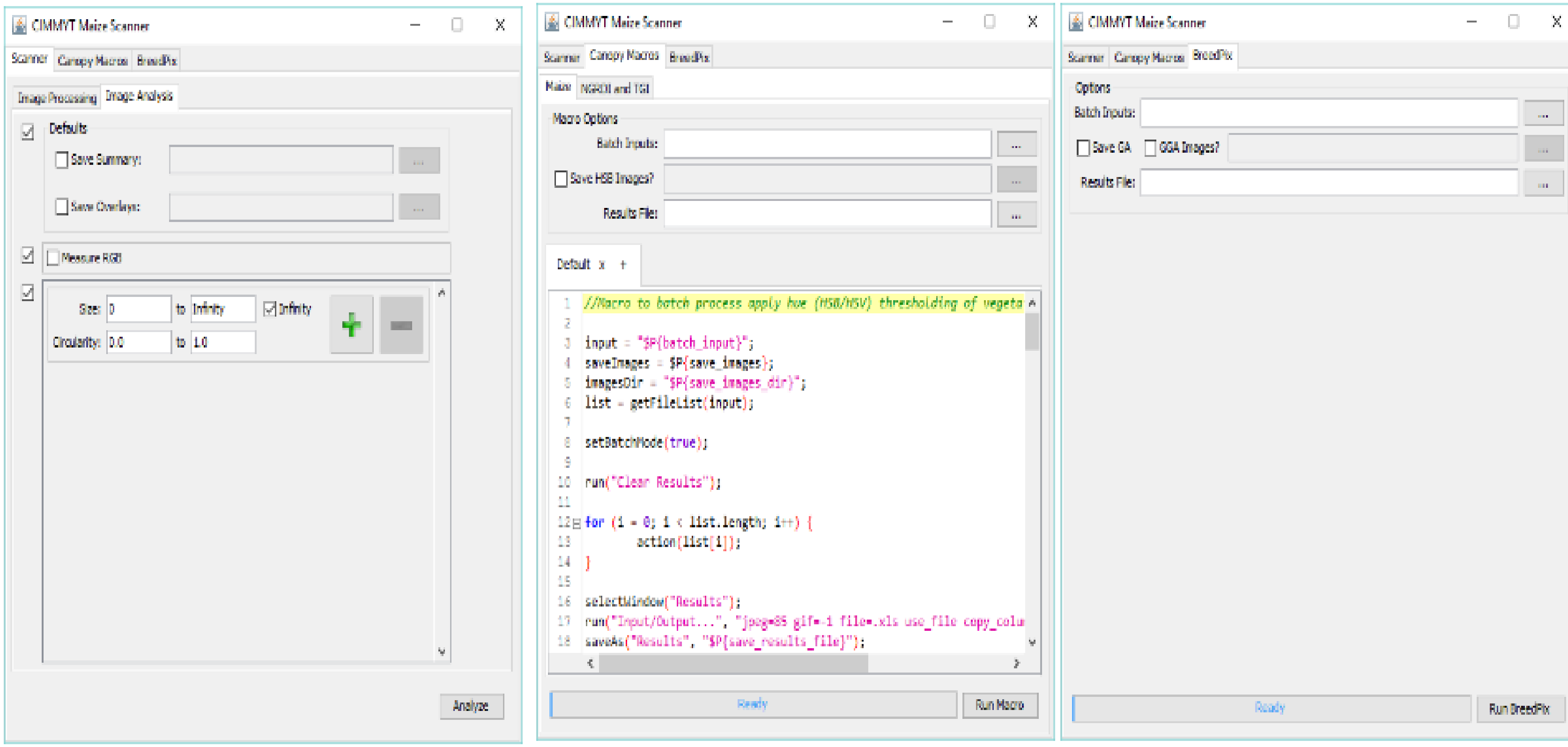
Summary

- Resource (radiation, water, nitrogen..) uptake and use efficiency are paramount to increase GY and adaptation
- A wide array of remote sensing techniques are already available
- Low-cost methodological approaches make high-throughput field phenotyping feasible
- Spatial variability may be monitored with remote sensing techniques
- Quality management of the field trials is required


Sensors
• Spectral
• Thermal
• Digital
Flight plan software
• 'GPS Positioning'
• 'Flight control'
• Telemetry
Aerial platform
• Payload
• Cost
• Safety
Lab. analysis - NIRS
Field variability
'Crop variability'
'Variation in biomass'
'Experimental layout'
Phenotyping
• Biomass
• Senescense
• 'Plant water status'
• 'Disease incidence'
Environmental data
• Meteorological
• Soil
Data processing
Genomic data
Data analysis

HTPP data processing tools, Open-source plug-ins, FIJI (Fiji is Just ImageJ)

Data collection as a bottleneck is over. In the age of BIG DATA, too much data needs new tools in order to overcome the data-to-decision obstacles.



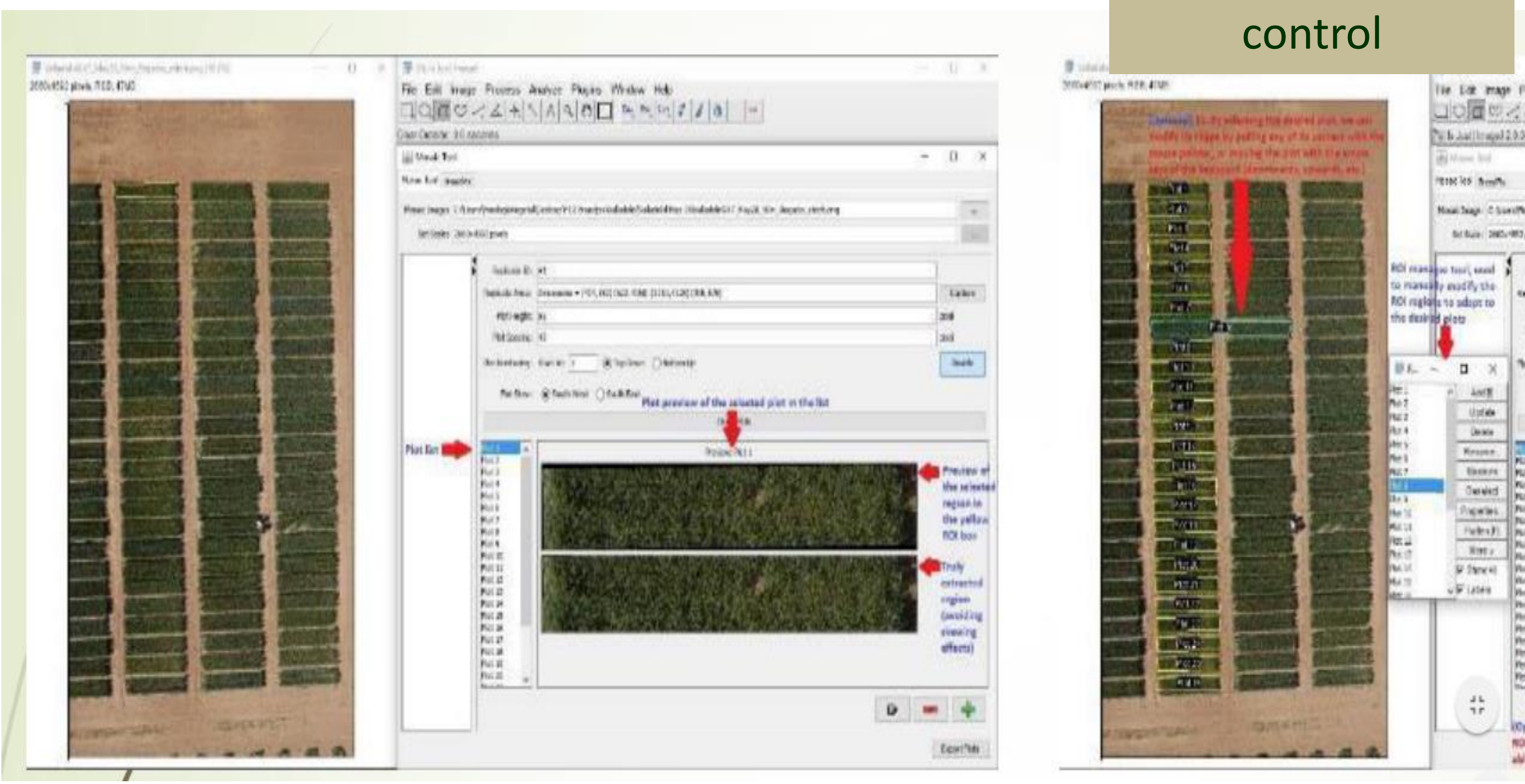
CIMMYT Maize Scanner for RGB field-based phenotyping (released at http://github.com/george-haddad/CIMMYT)

Calculates a number of RGB based indexes for estimating disease impacts, crop vigor, LAI, biomass at the leaf and canopy scale, including Breedpix (GA and GGA), Triangle Greeness Index (TGI), and Normalized Green Red Difference Index (NGRDI)

MosaicTool (Plugin for FIJI)

Semi-automatic image segmentation for UAV plant phenotyping studies.

Allows for the extraction and processing of ~1000 plots per hour with quality control

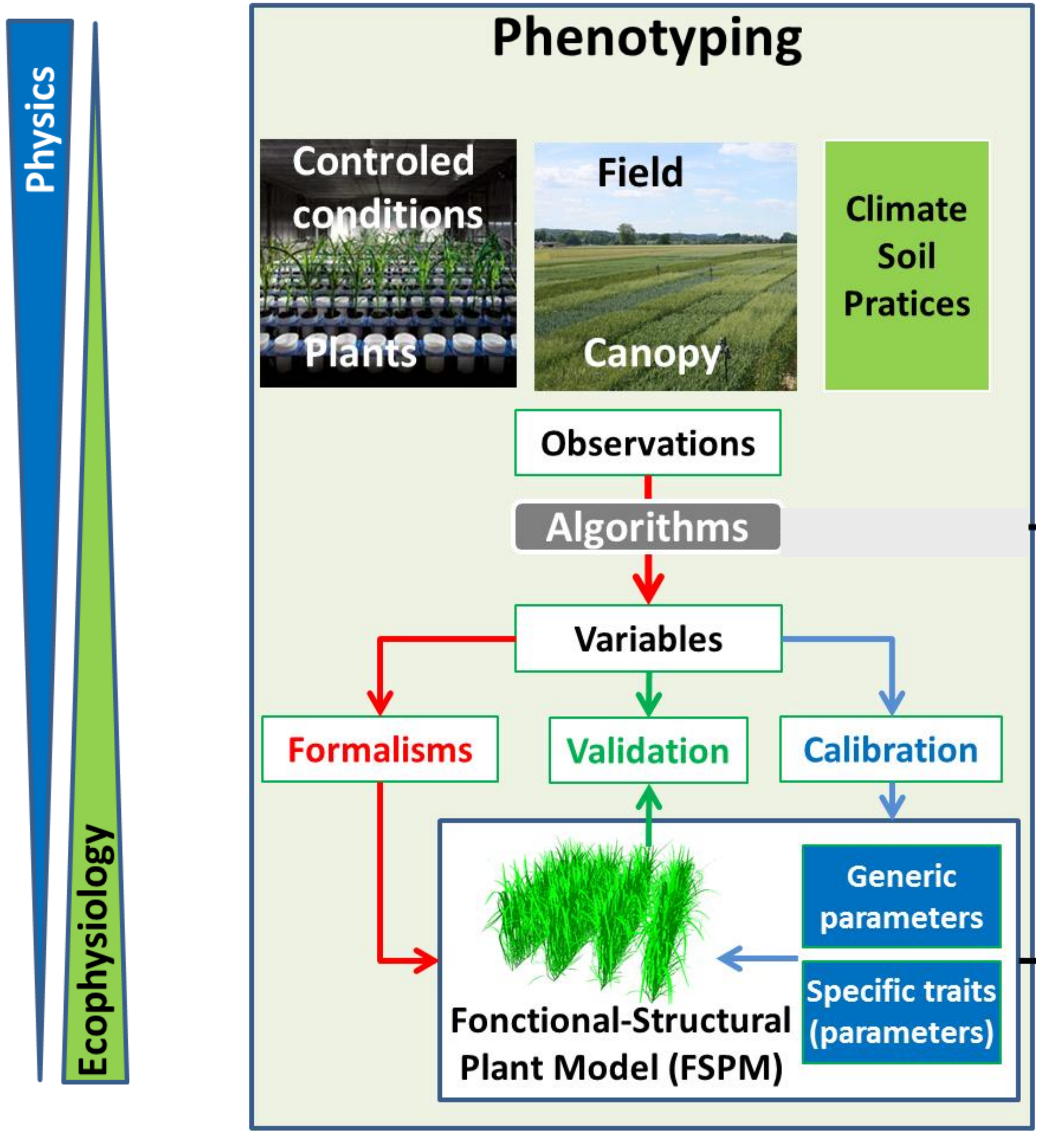


Future bottlenecks

Data explosion

- We generate far too much data to handle manually
- Simple summary statistics do not suffice
- Advanced analysis tools, models, selection indexes are required

Phenotyping will contribute and benefit from crop modeling (FSPMs)



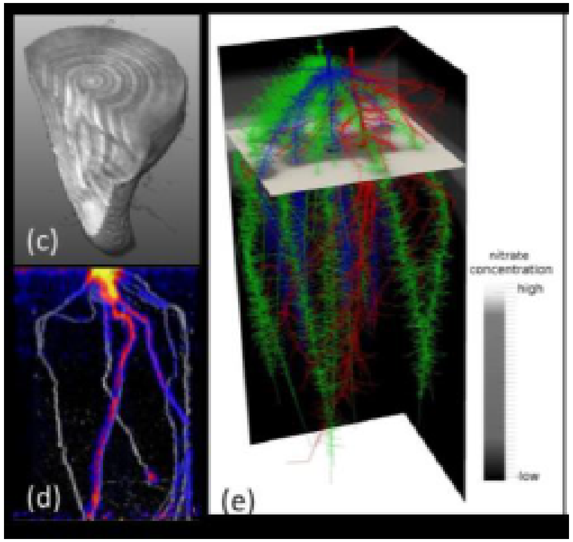
State-of-the-Art Phenotyping

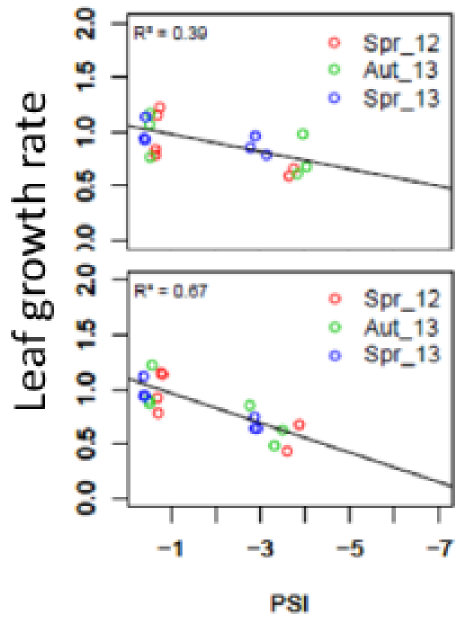
Modelling

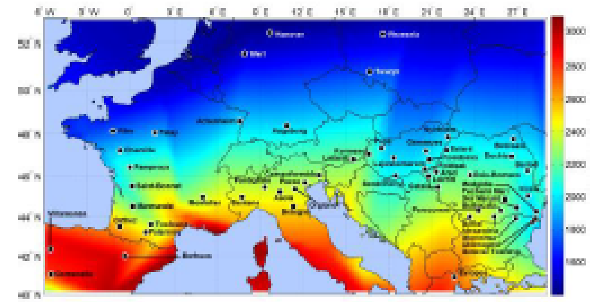
Disentangling complex traits

Genetic analysis of complex traits

Crop – climate optimisation





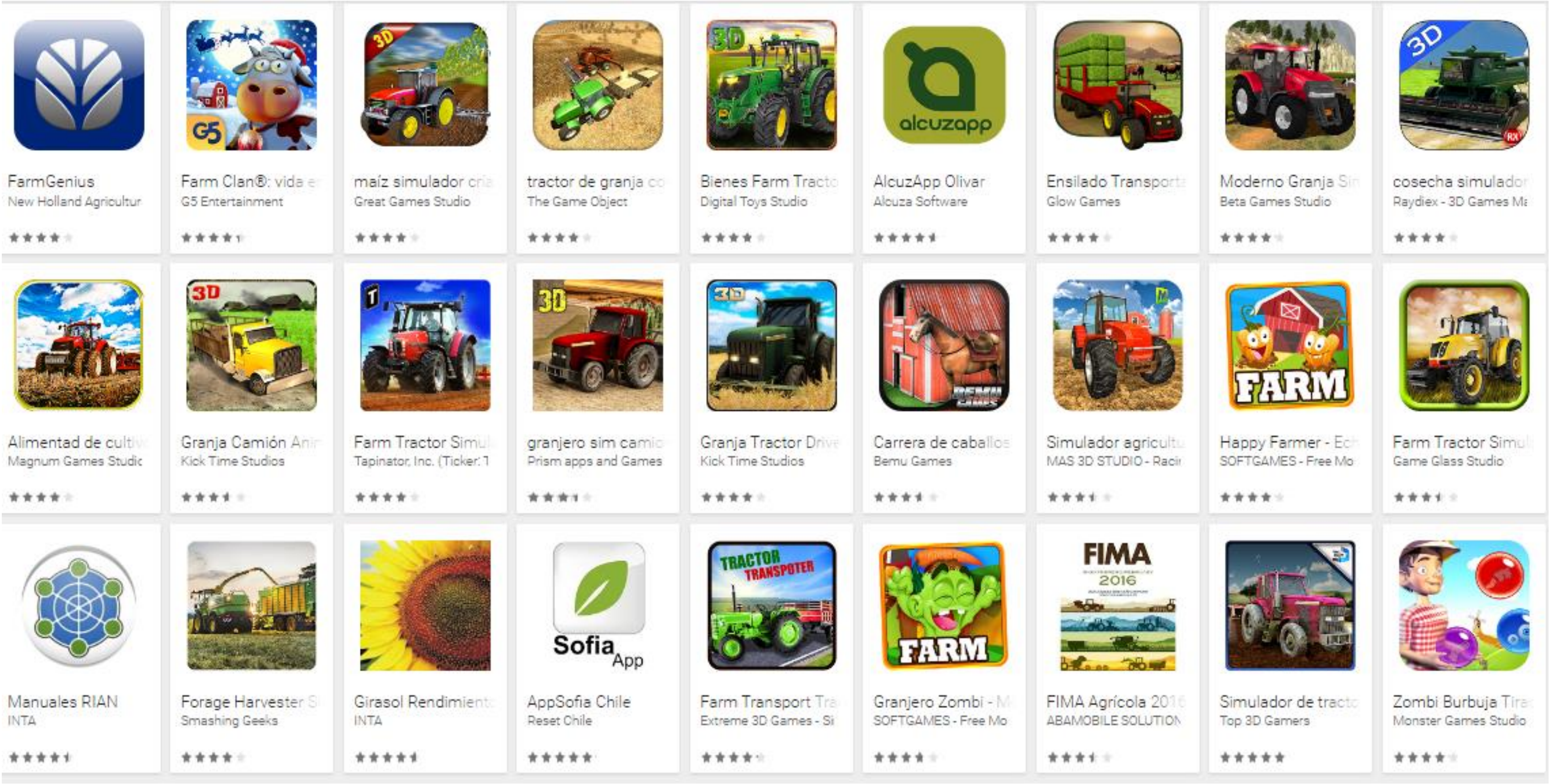


What is the relationship between root structure and nutrient use efficiency?

What is the sensitivity of leaf growth to drought?

Which genotype would work best in which environment scenario?

Mobile Apps

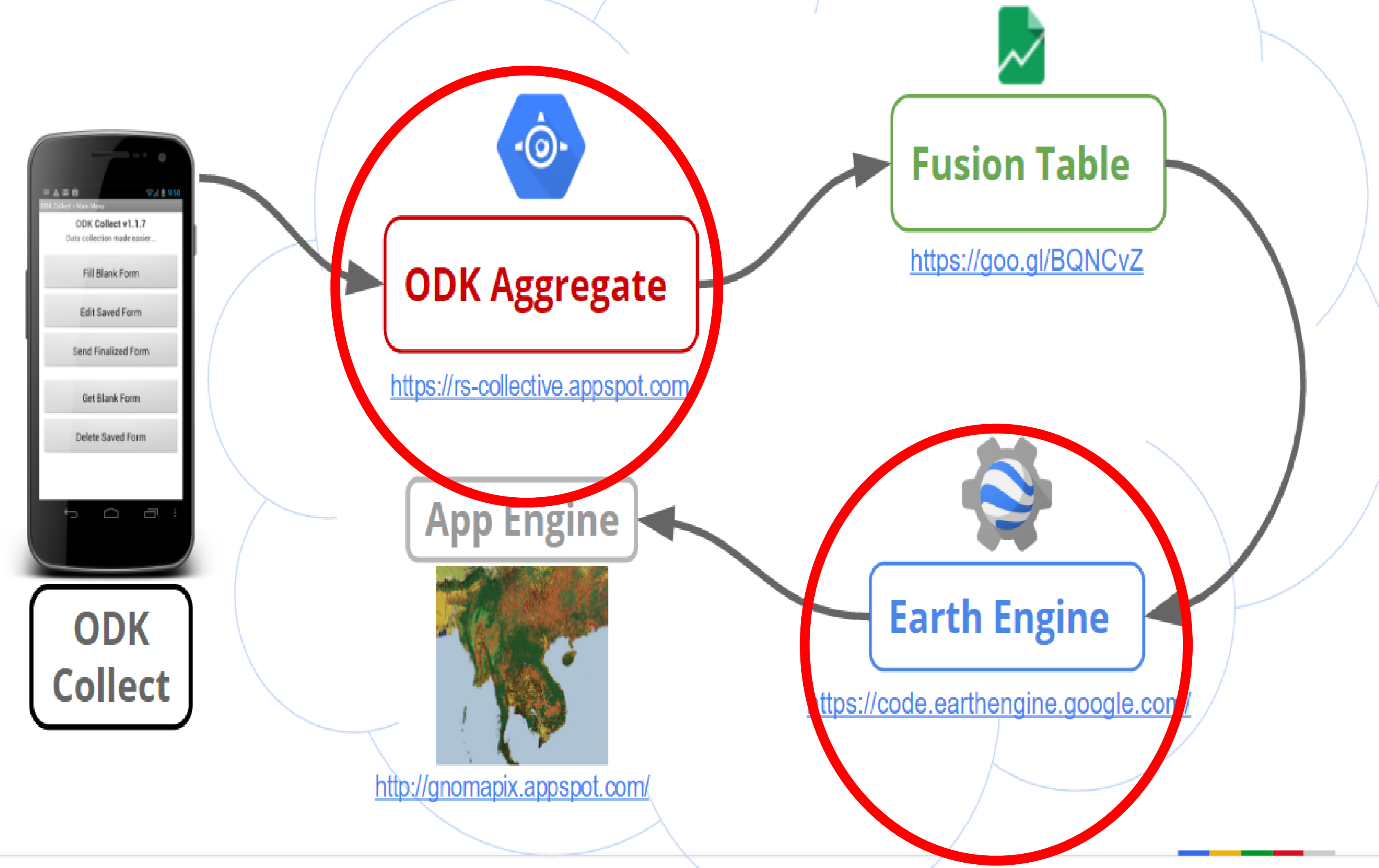


FarmGenius
New Holland Agricultur
Farm Clan®: vida e
G5 Entertainment
maíz simulador cria
Great Games Studio
tractor de granja co
The Game Object
Bienes Farm Tracto
Digital Toys Studio
AlcuzApp Olivar
Alcuza Software
Ensilado Transporta
Glow Games
Moderno Granja Si
Beta Games Studio
cosecha simulador
Raydiex - 3D Games Ma
Alimentad de cultiv
Magnum Games Studio
Granja Camión Ani
Kick Time Studios
Farm Tractor Simul
Tapinator, Inc. (Ticker: T
granjero sim camio
Prism apps and Games
Granja Tractor Drive
Kick Time Studios
Carrera de caballos
Bemu Games
Simulador agricultu
MAS 3D STUDIO - Racir
Happy Farmer - Ech
SOFTGAMES - Free Mo
Farm Tractor Simul
Game Glass Studio
Manuales RIAN
INTA
Forage Harvester S
Smashing Geeks
Girasol Rendimient
INTA
AppSofia Chile
Reset Chile
Farm Transport Tra
Extreme 3D Games - Si
Granjero Zombi - M
SOFTGAMES - Free Mo
FIMA Agrícola 2016
ABAMOBILE SOLUTION
Simulador de tracto
Top 3D Gamers
Zombi Burbuja Tira
Monster Games Studio

Open Data Kit (ODK)

- There's an opportunity for mobile and cloud technologies to enable timely and efficient data collection.
- Open Data Kit (ODK) is a suite of tools that enable efficient and timely data collection on cell phones. ODK is designed to let users own, visualize, and share data without the difficulties of setting up and maintaining servers. The tools are easy to use, deploy, and scale. They also go beyond open source - they're based on open standards and supported by a larger community.

Mobile to Cloud pipeline



Google Earth Engine

https://opendatakit.org/

Shawn C. Kefauver

Acknowledgements

AGL2016-76527-R

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

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INSTITUTO TECNOLÓGICO AGRARIO
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Consejería de Agricultura y Ganadería

iCrea
INSTITUCIÓ CATALANA DE RECERCA I ESTUDIS AVANÇATS

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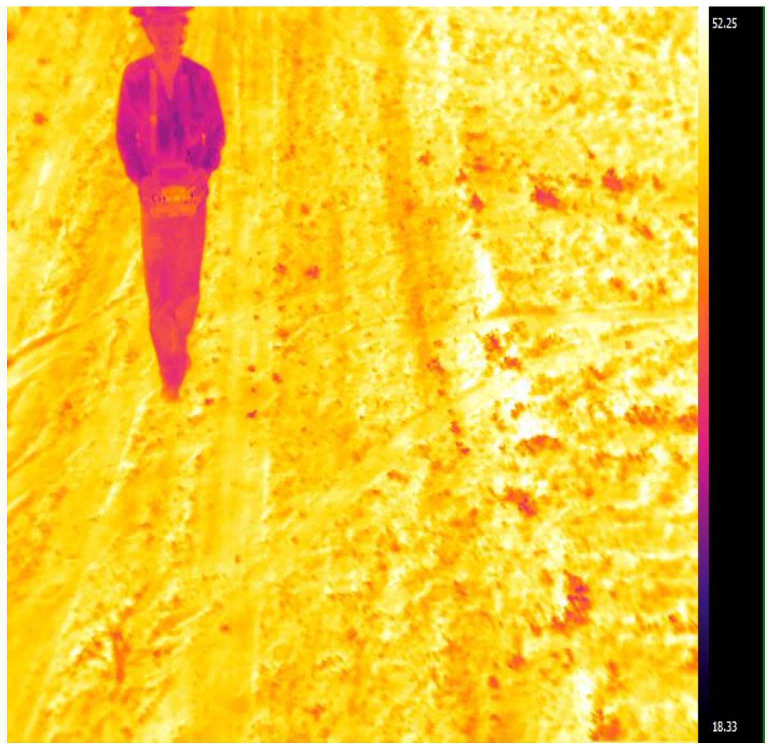
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria



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Jordi Bort
Maria D. Serret

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M. Zaman-Allah
B.M. Prasanna

- University of Barcelona, Faculty of Biology, Integrative Crop Ecophysiology Group
- **http://integrativecropecophysiology.com,**


thank you very much
mange tak!
tack så mycket!
tusen takk!