RGB low-cost imaging for plant phenotyping: index calculation and analysis

Training programme on Affordable Phenotyping
Matera-Metaponto, Italy 18-21 April 2023

EMPHASIS

EMPHASIS-GO is supported by the European Union (Grant Agreement: 101079772).

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Sustainable Field Crops Program

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IRTA
RECERCA I TECNOLOGIA AGROALIMENTÀRIES

Introducing myself…

Adrian Gracia-Romero

- *2017-2021*

UNIVERSITAT DE BARCELONA

agrotecnio

PhD - "Advances in high throughput and affordable phenotyping for adapting maize and wheat to climate change"

Integrative Crop Ecophysiology Group (Evolutionary Biology, Ecology and Environmental Sciences Department), Universitat de Barcelona, Barcelona, Spain

- *2019*

THE UNIVERSITY OF MELBOURNE

Hyperspectral Remote Sensing & Precision Agriculture Laboratory (Univ. of Melbourne, Australia)

- *2022-*

IRTA RECERCA I TECNOLOGIA AGROALIMENTÀRIES

Postdoctoral Researcher in Plant Physiology and Crop Phenotyping

Sustainable Field Crops Program at Institute of Agrifood Research and Technology (IRTA) – Lleida, Spain


Science
to feed
the future
IRTA

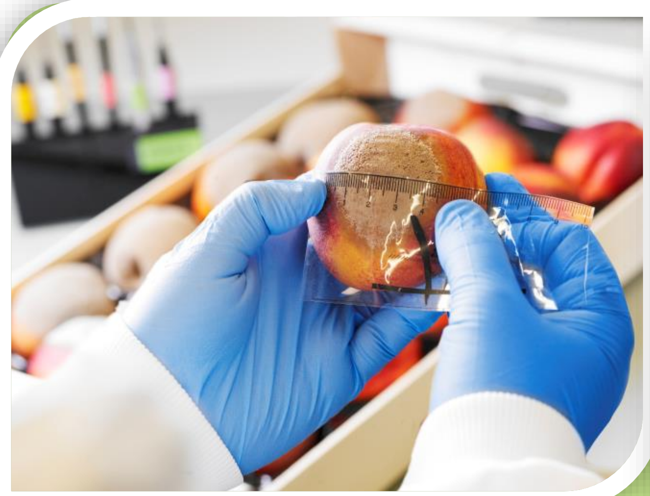
We are the scientific reference in the agri-food field in Catalonia.

We are the driving force behind innovation and knowledge transfer in the agri-food industry.

We offer solutions to transform the agri-food systems into more resilient and sustainable systems and contribute to:

- ✓ **mitigating climate change,**
- ✓ **preserving biodiversity and**
- ✓ **using natural resources more efficiently.**

Research areas



Plant Production

- Efficient Use of Water in Agriculture
- Fruit Production
- Genomics and Biotechnology
- Postharvest
- Sustainable Field Crops
- Sustainable Plant Production

Animal Production

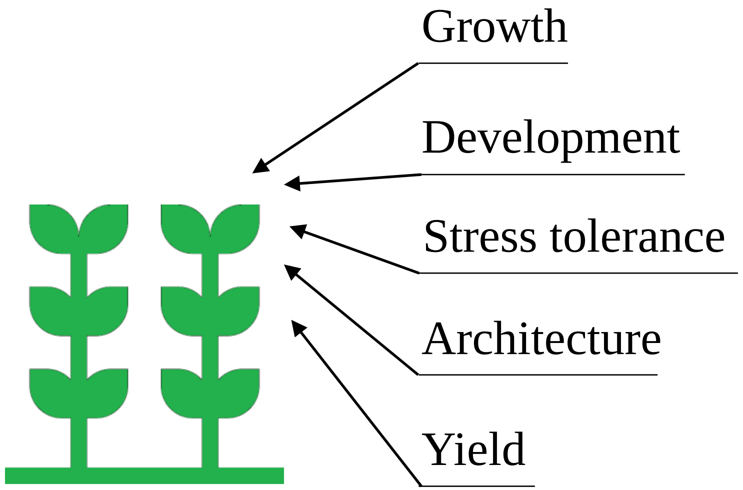
- Animal Breeding & Genetics
- Animal Health
- Animal Nutrition
- Animal Welfare
- Aquaculture
- Sustainability in Bioystems
- Marine and Continental Water
- Ruminant Production

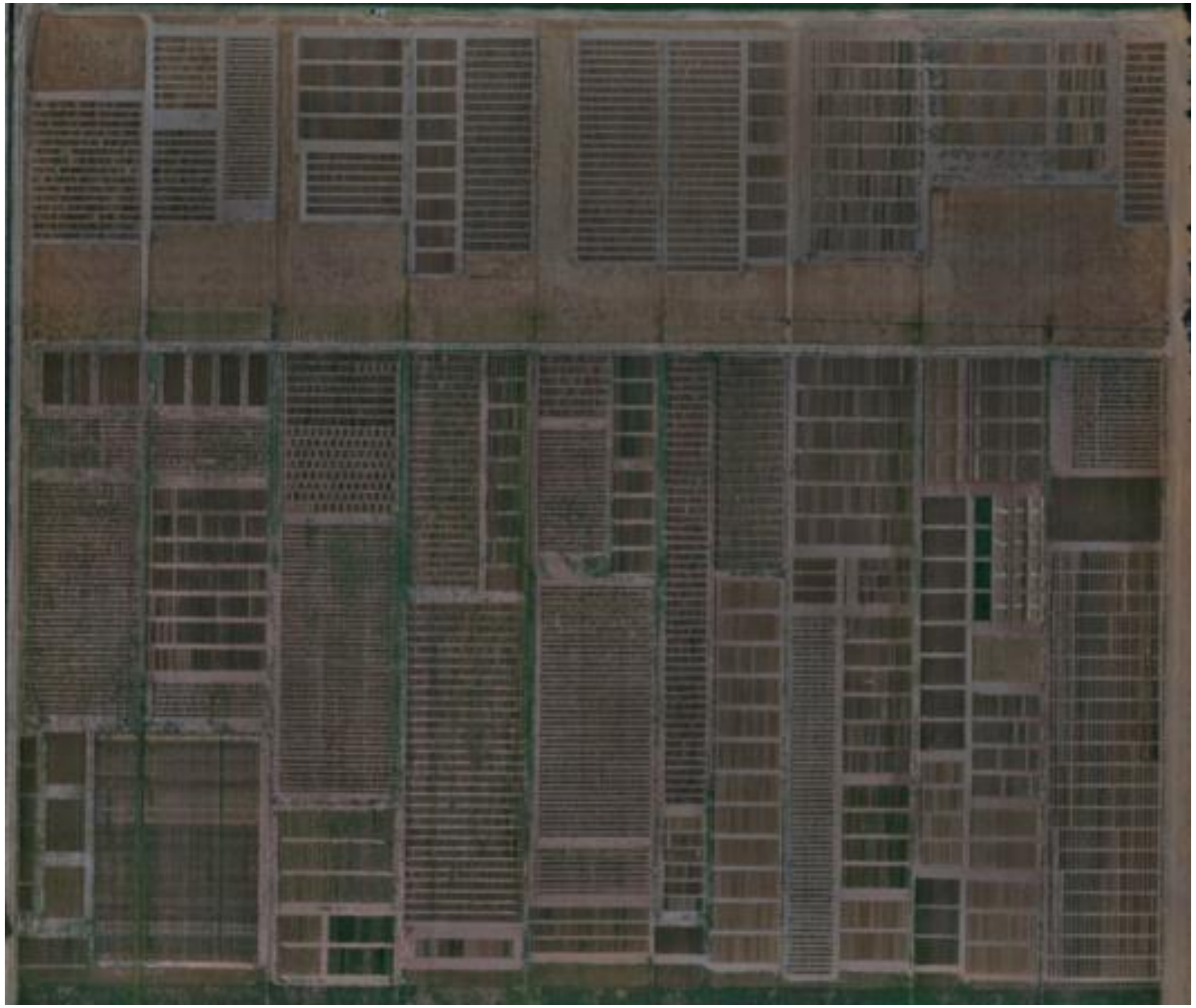
Food Industries

- Food Safety and Functionality
- Food Quality and Technology

Agro-food economics

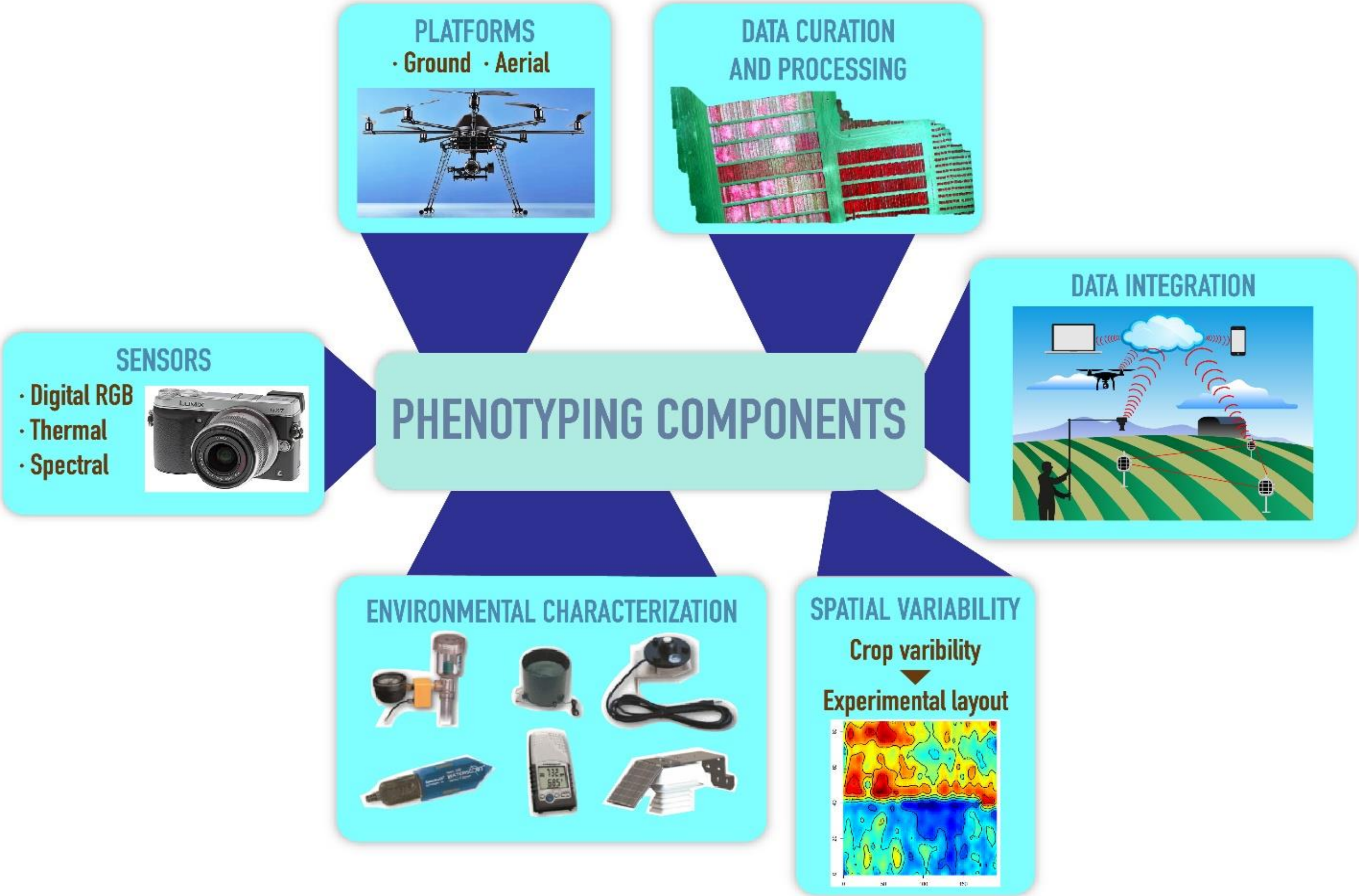
Current context and challenges





Current context and challenges

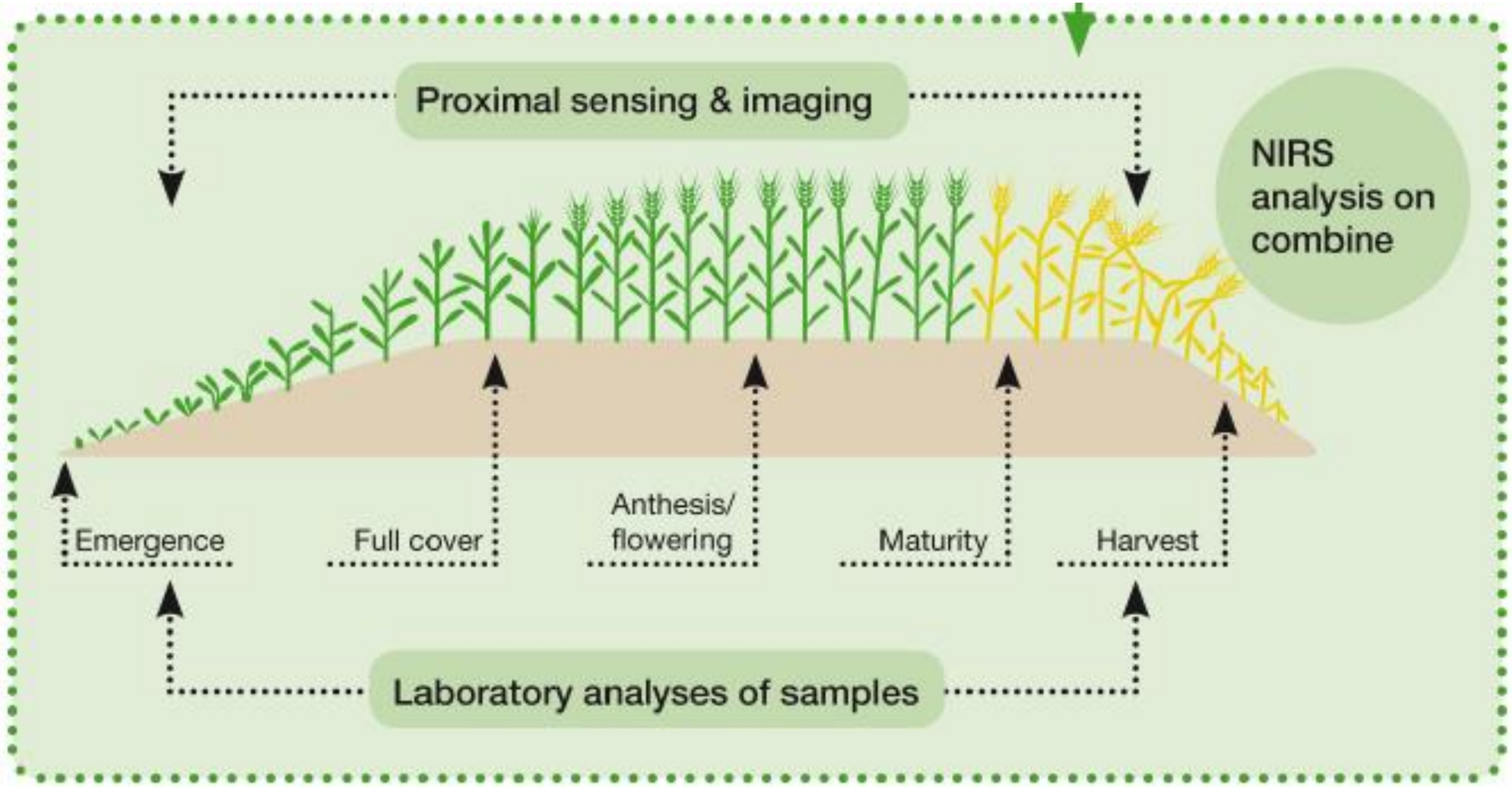
How to assess large-scale trials in a rapid and cost-effective way?



Araus & Kefauver, 2018 *Curr. Opin Plant Biol.*

Closing gaps through plant phenotyping

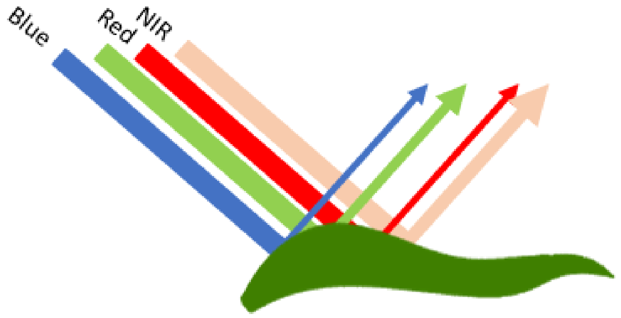
The role of remote sensing

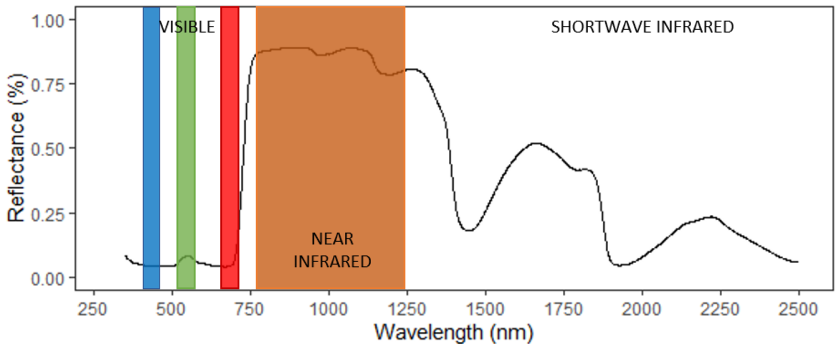


Araus & Cairns 2014, *Trends Plant Sci.*

The role of remote sensing

Multispectral/hyperspectral sensors



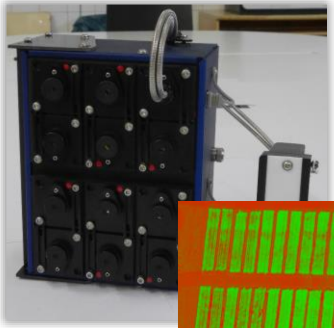


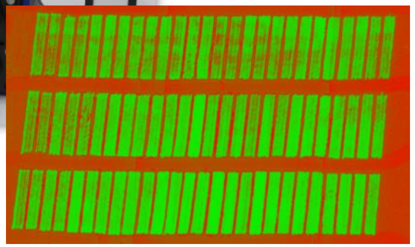
- Vegetation cover and vigor

$$Normalized\ Difference\ Vegetation\ Index = \frac{(R840nm - R670nm)}{(R840nm + R670nm)}$$

- Photosynthetic capacity

$$Photochemical\ Reflectance\ Index = \frac{(R531nm - R570nm)}{(R531nm + R570nm)}$$

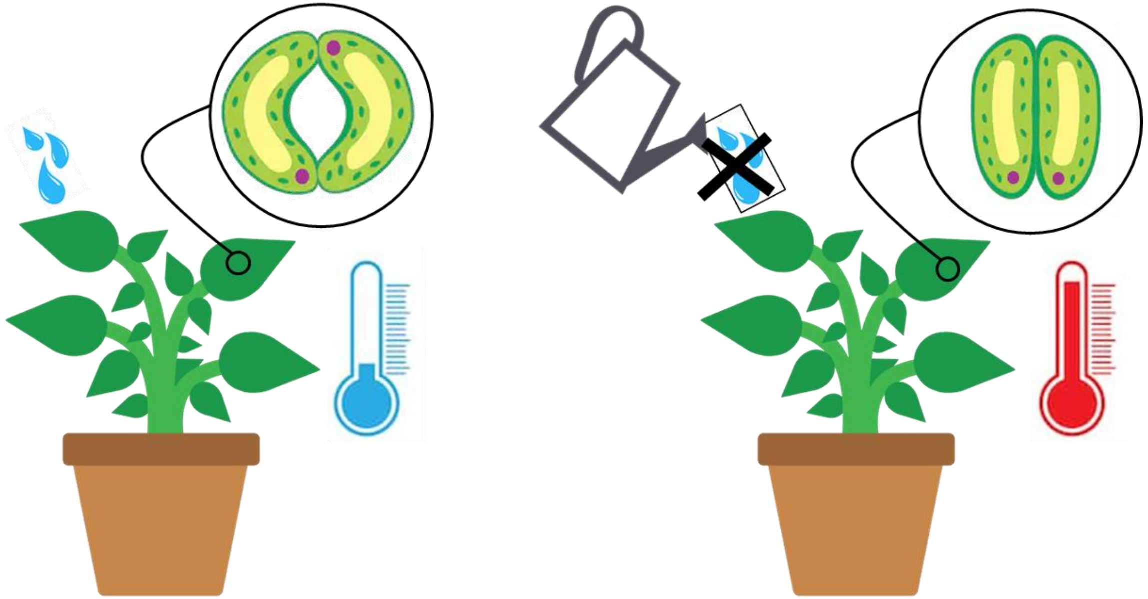


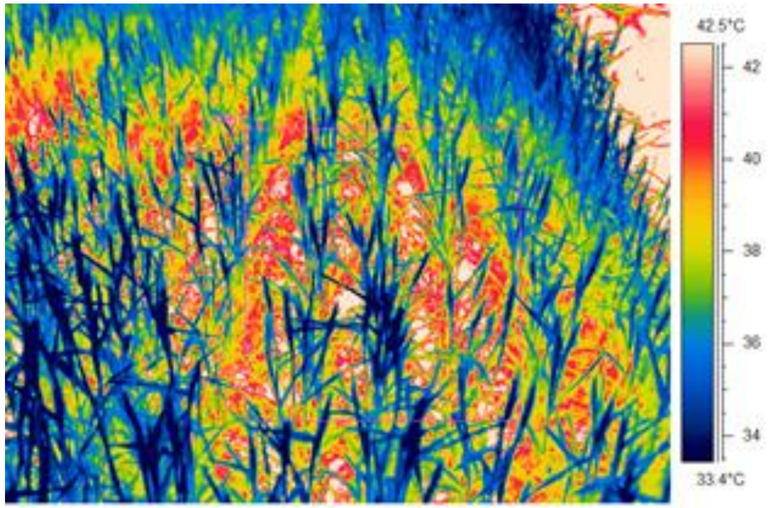


The role of remote sensing

Transpiration is one of the main factors reducing plant's temperature

Canopy temperature as an indicator of crop water status



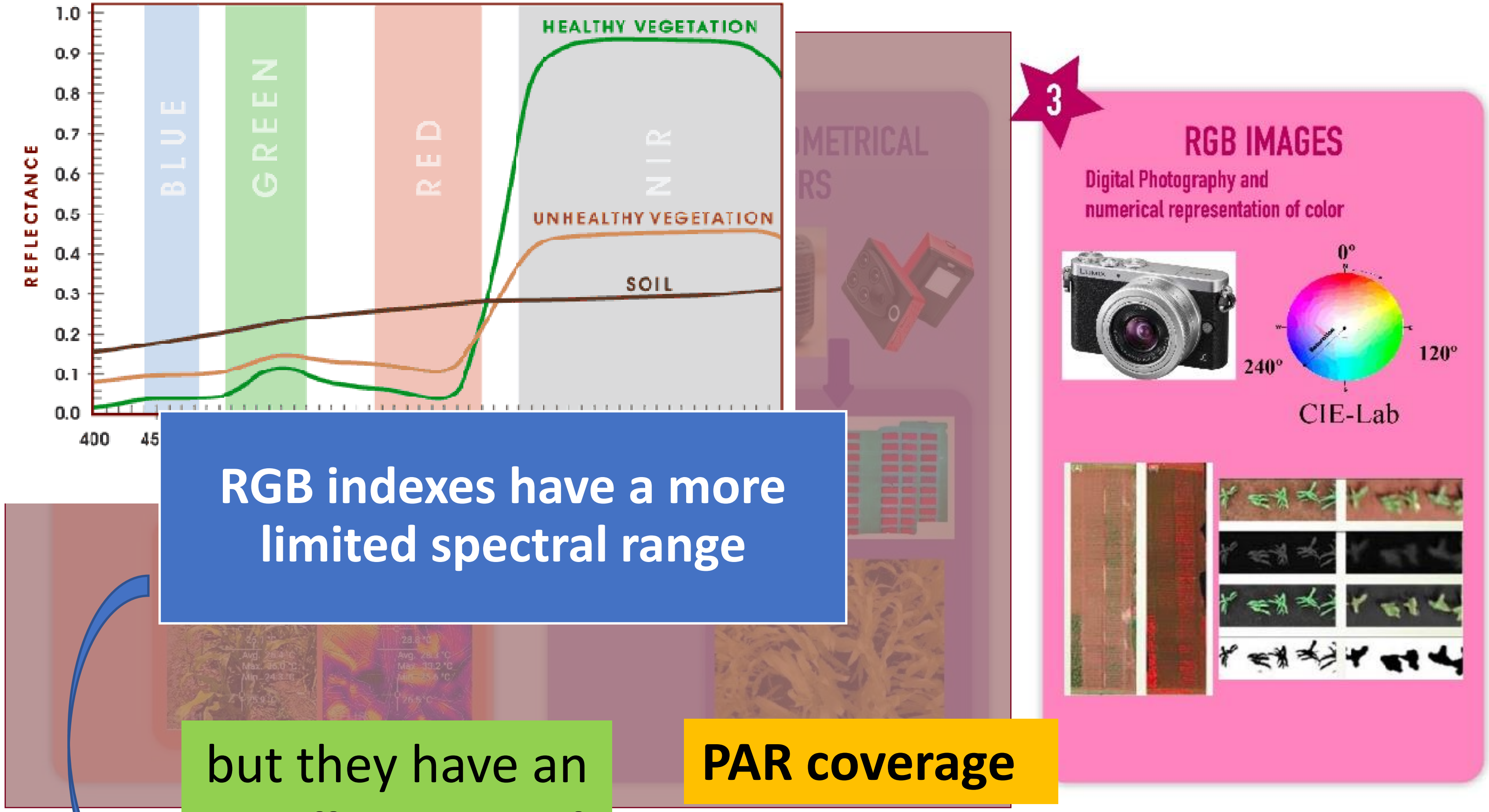


Wheat plot from an experiment in Algeria

The role of remote sensing

Araus & Kefauver, 2018 *Curr. Opin Plant Biol.*

The role of remote sensing



RGB indexes have a more limited spectral range

but they have an **excellent spatial resolution (cm – mm)**

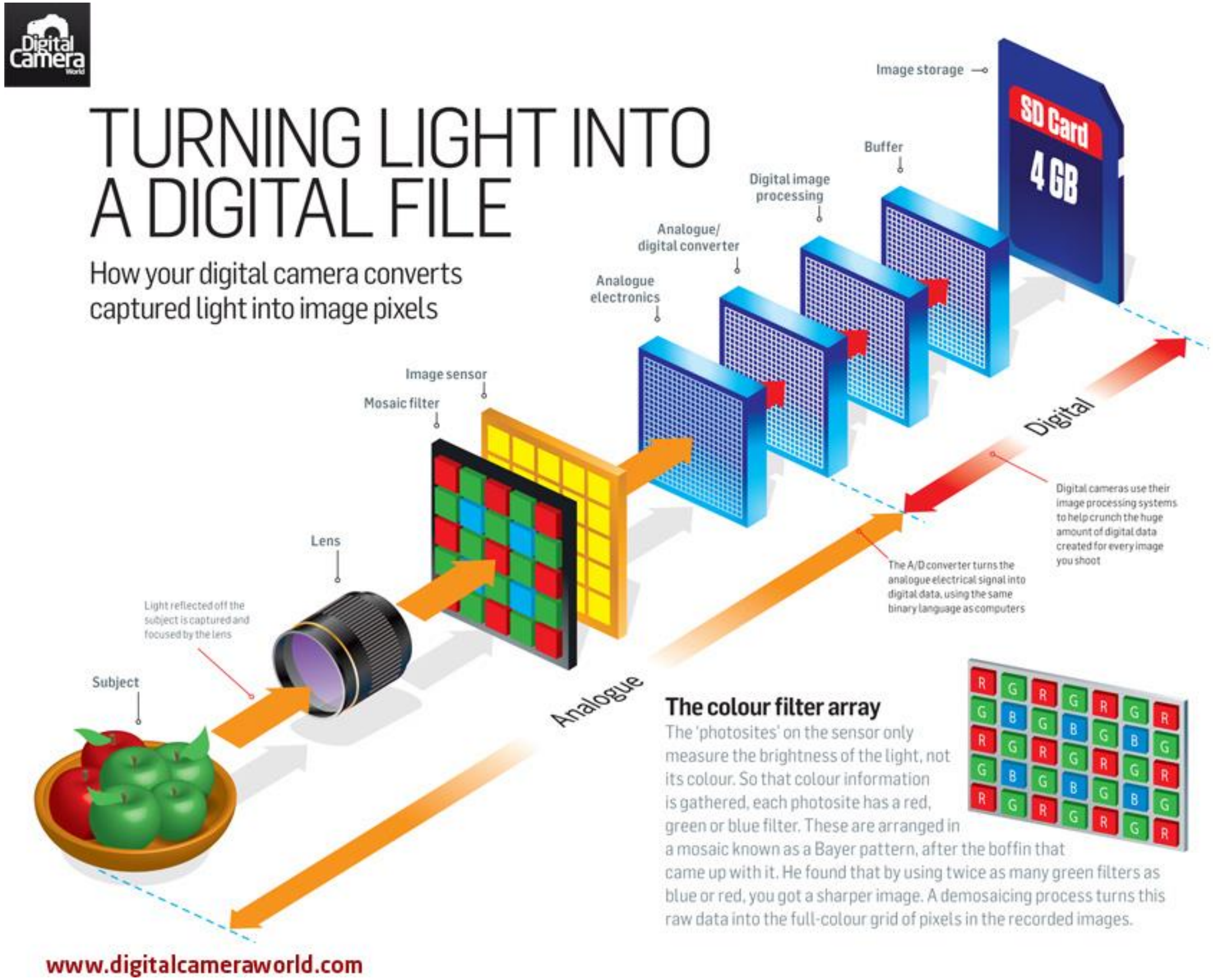
PAR coverage

Excellent color calibration

Araus & Kefauver, 2018 *Curr. Opin Plant Biol.*

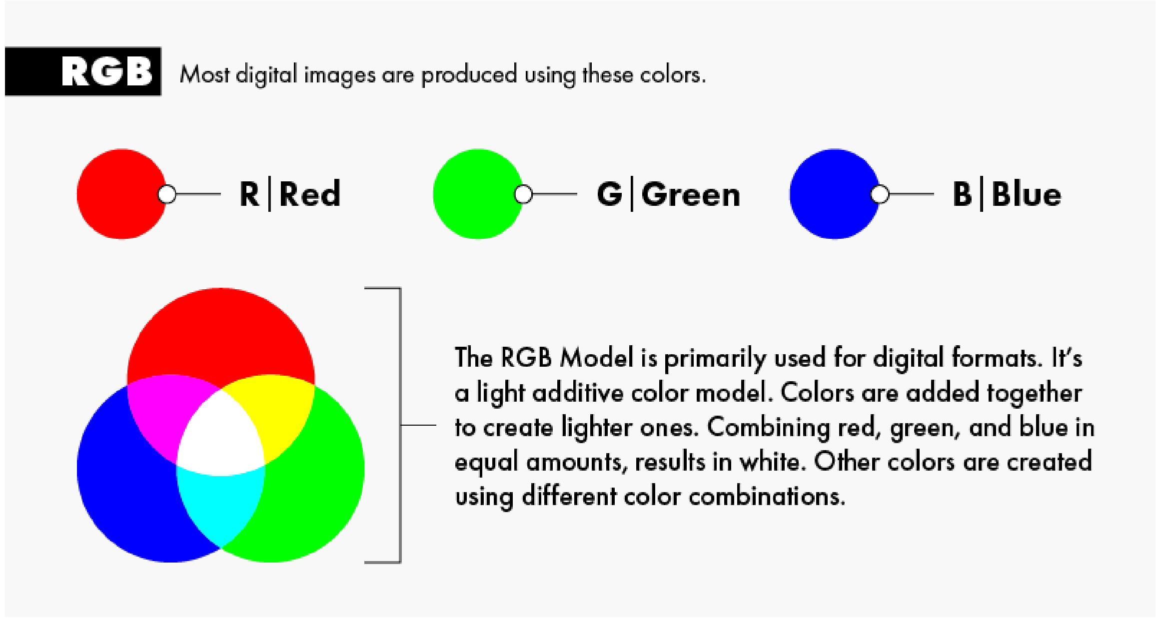
Low-cost phenotypical approaches exist

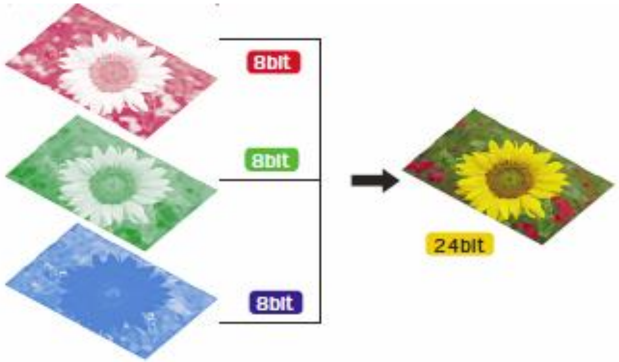
RGB vegetation indices



Low-cost phenotypical approaches exist

RGB vegetation indices

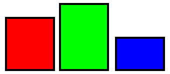




Low-cost phenotypical approaches exist

RGB vegetation indices

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

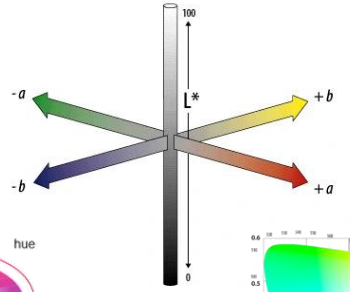


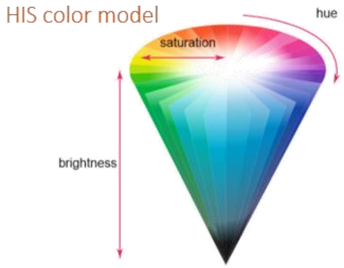
•RGB: Red, Green and Blue

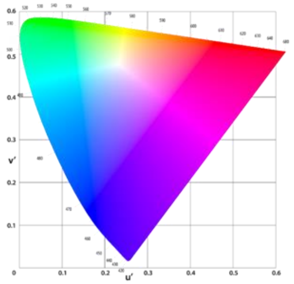
related with color reproduction by computer screens, etc.

•Hue, Intensity, Saturation model
•CIE-lab//CIE-Luv

Practical for image analysis

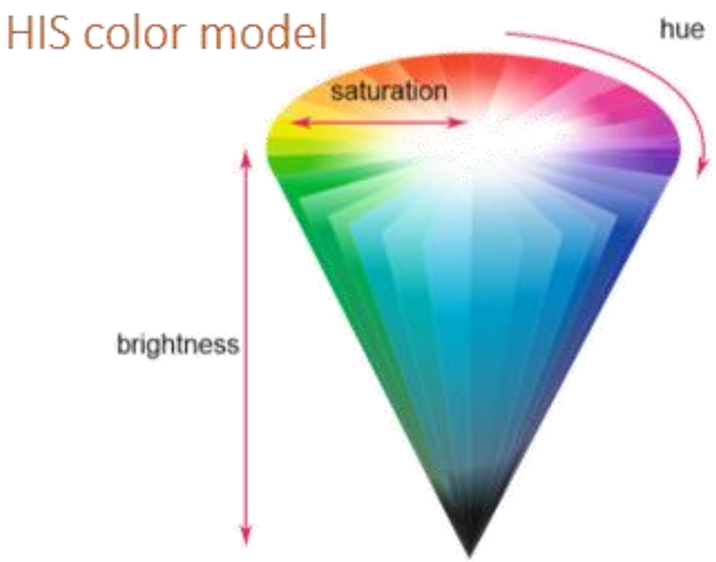




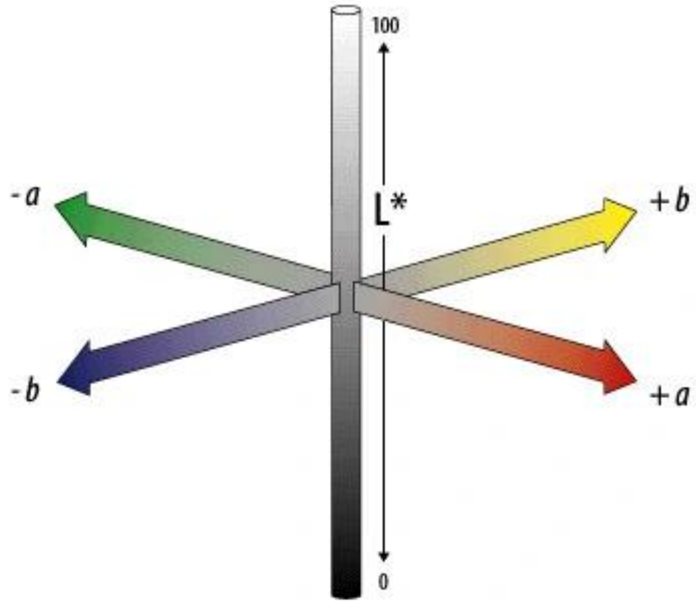


RGB imaging

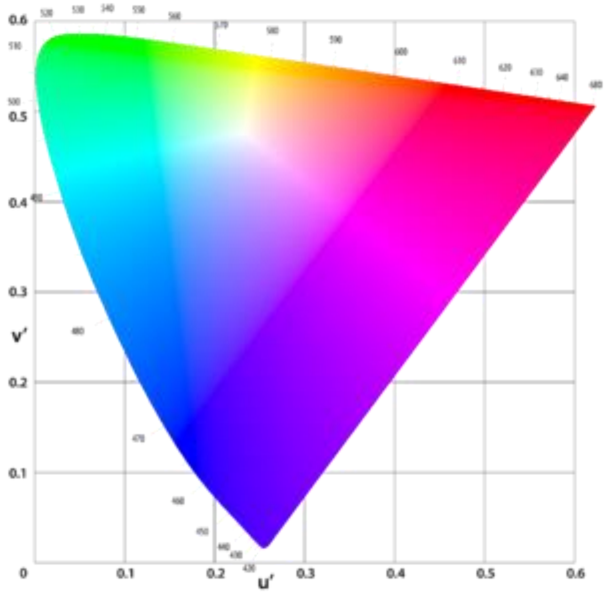
HIS color model



CIE-Lab color model

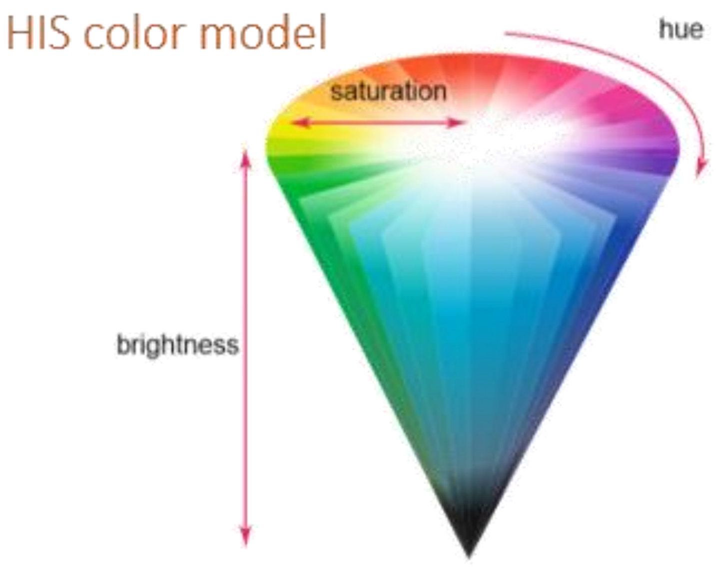


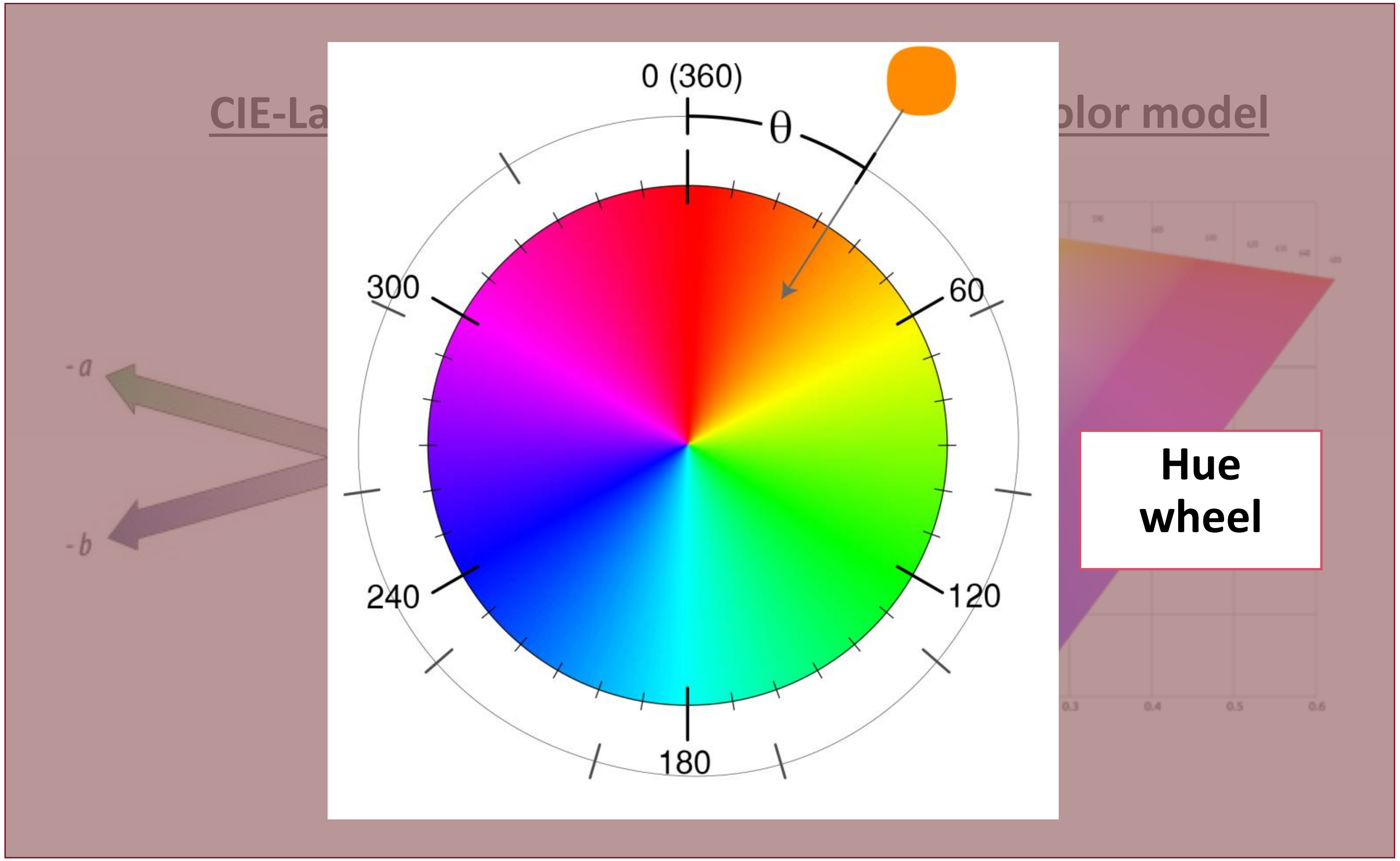
CIE-Luv color model



RGB imaging

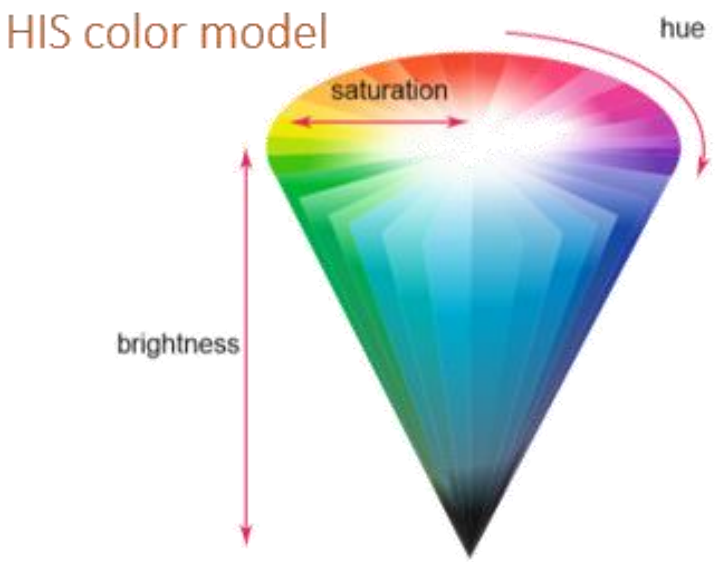
HIS color model



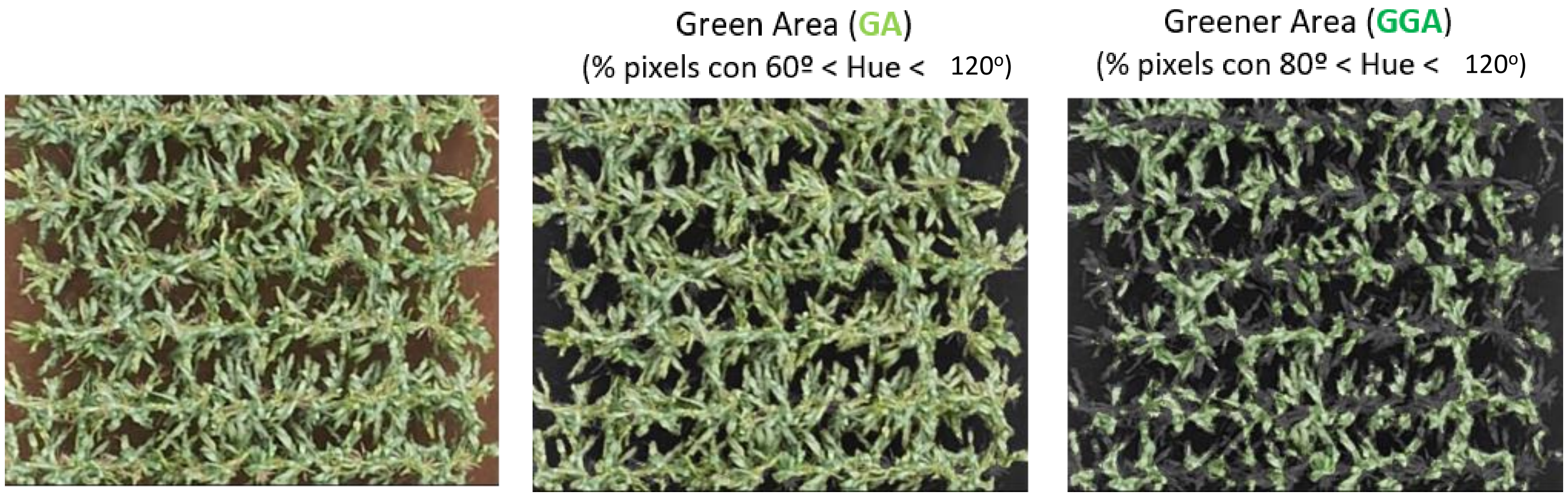


Low-cost assessment of vegetation indices

RGB imaging



Tomato plants experiment (University of Melbourme, Australia 2019)



Maize under different N regimens experiment (CIMMYT, Zimbabwe 2017)

Low-cost asses

RGB ima

HIS color model

- **Using FIJI to select and measure images based on pixel colour counts. Manual calculation of the Green Area index based on the parameter Hue.**

1. Open the image. Must be in format of 24 bits (RGB Colour, 8-bits x 8-bits x 8-bits = 24).

2. Converts the type of image: Image - > Type - > HSB Stack

3. Separate images by selecting Images - > Stacks - > Stack to Images.
4. Close the images of Saturation and Brightness.

5. Select the image's Hue (hue). Apply the Threshold: Go to Image - > Adjust - > Threshold.

6. To calculate the GA we have to select the Hue values according to the values of green and green/yellow indicated by the HSB model, that are values of 0 to 360, but we have pictures of 8-bit values from 0 to 255, because we have to do some calculations...

GA = Green Area (360: pixels with 60 < Hue < 120; for 255: 42.5 < Hue < 85)
GGA = Greener Area (360: pixels with 80 < Hue < 120 360 are 57 < Hue < 85)

7. Select the area of interest. Go to Edit -> Selection -> Create Selection.

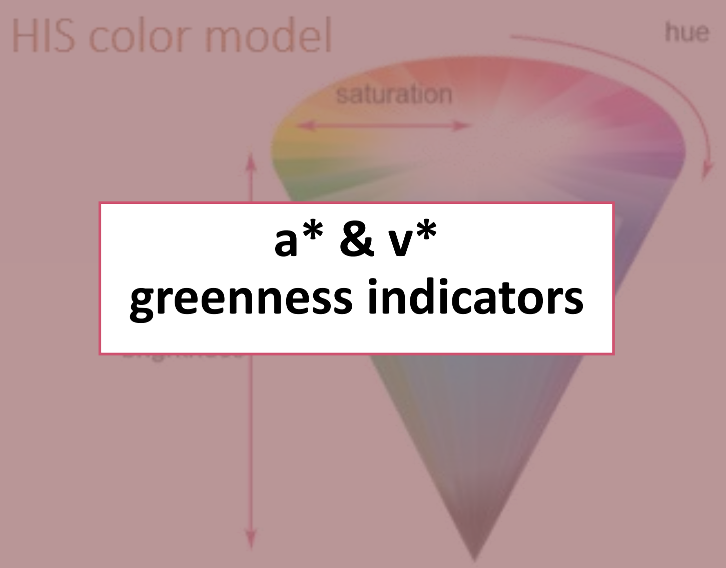
8. Measure the area of interest. Go to Analyse - > Measure.

rea (GGA)
0º < Hue < 120º)

Maize under different N regimens experiment (CIMMYT, Zimbabwe 2017)

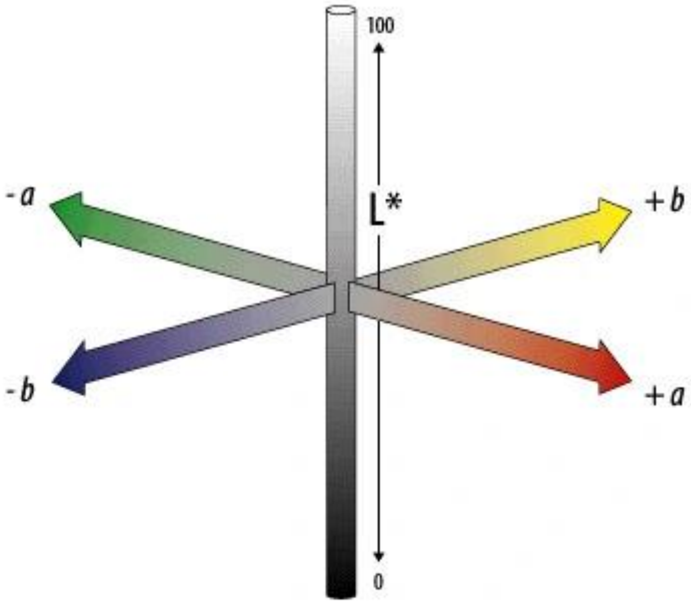
RGB imaging

HIS color model

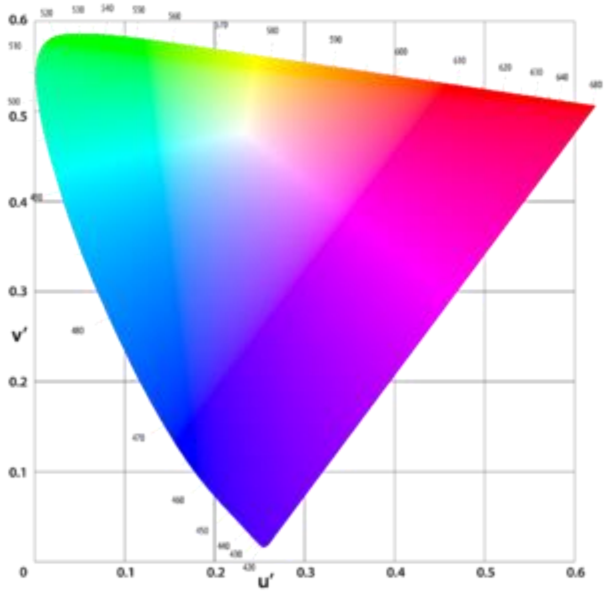


**a* & v*
greenness indicators**

CIE-Lab color model



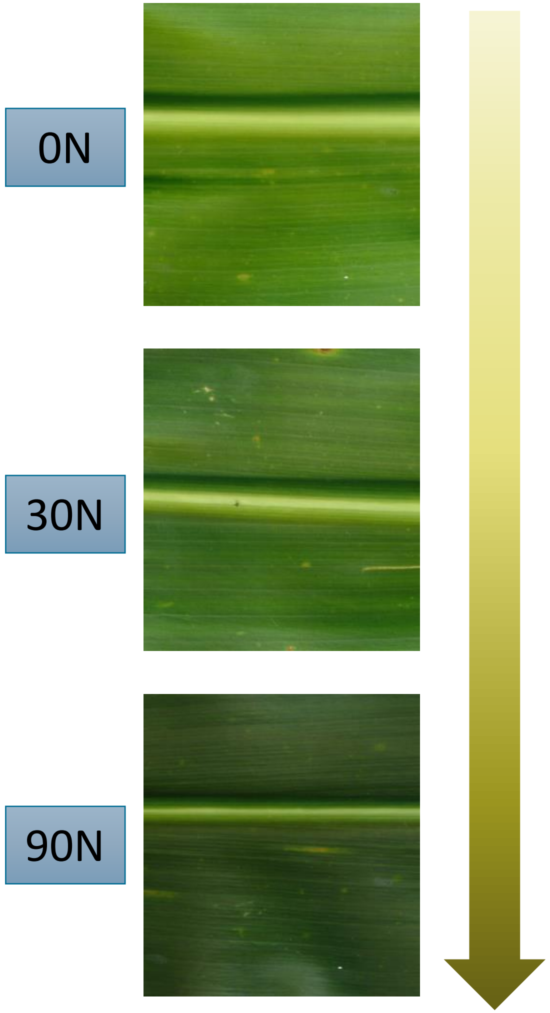
CIE-Luv color model

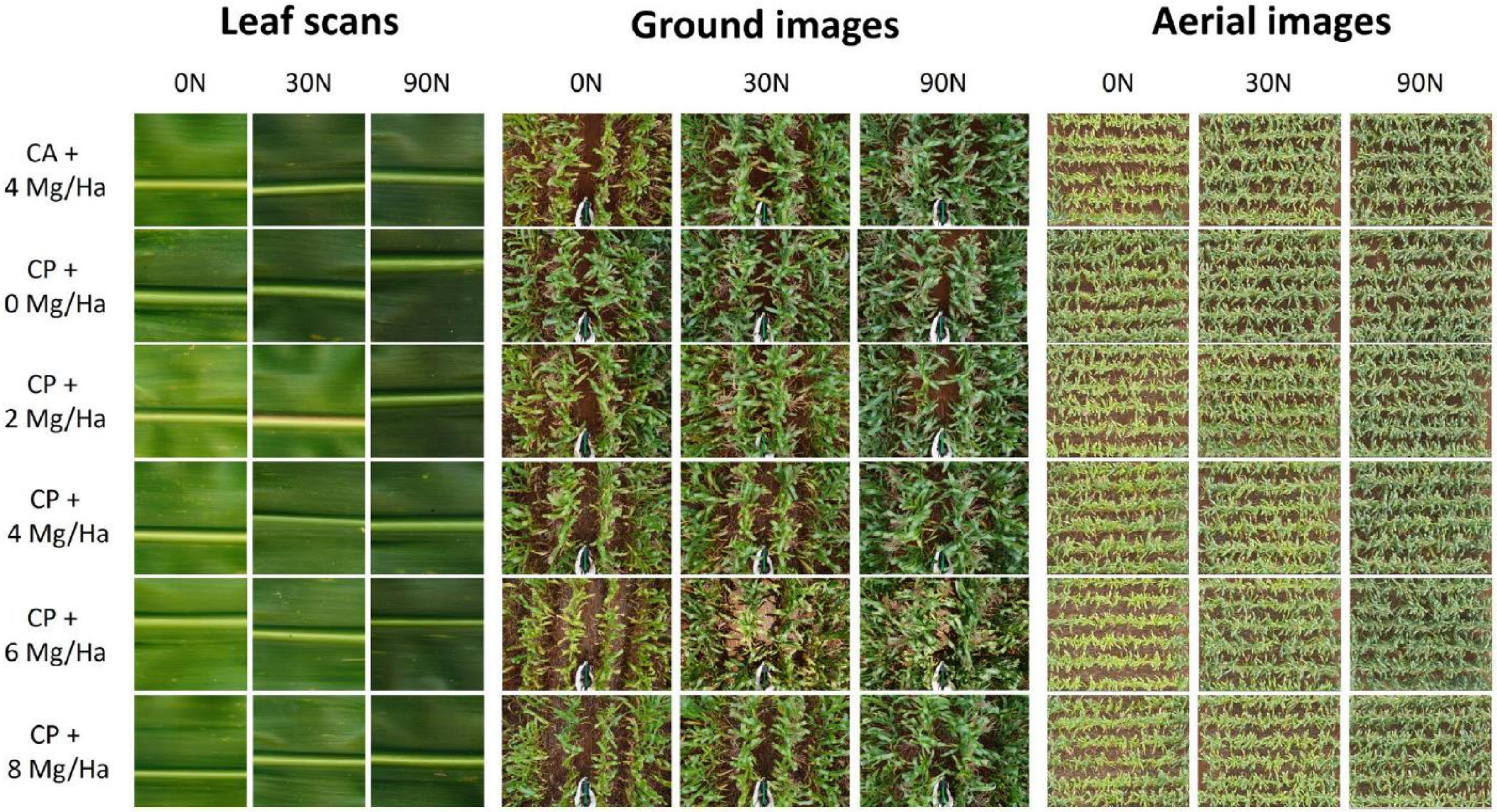


Low-cost assessment of vegetation indices

Gracia-Romero, 2019 *Scientific Reports*

RGB imaging



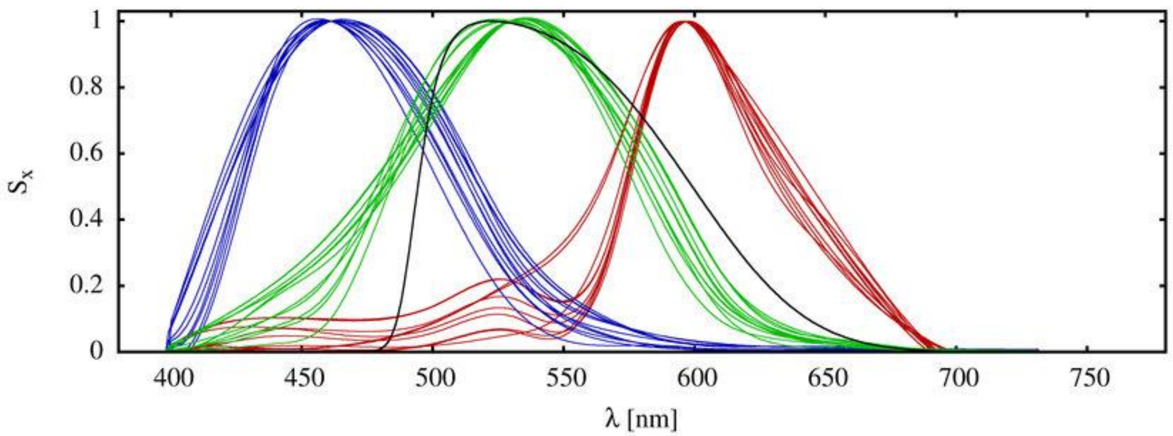


Darker green **More negative a*** → Higher chlorophyll content → Higher N content Higher yields

Maize under different N regimens experiment (CIMMYT, Zimbabwe 2017)

Low-cost assessment of vegetation indices

RGB imaging



Typical spectral sensitivity curves of commercial digital cameras with RGB bands.

Kolláth, 2020 *Journal of Quantitative Spectroscopy and Radiative Transfer*

VI Name	Equation	Reference
RGBVI	$(R_G{*}R_G) - (R_R{*}R_B)$ / $(R_G{*}R_G) + (R_R{*}R_B)$	Bendig et al. 2015
GLI	$(2{*}R_G - R_R - R_B)$ / $(2{*}R_G + R_R + R_B)$	Louhaichi et al. 2001
VARI	$(R_G - R_R)$ / $(R_G + R_R - R_B)$	Gitelson et al. 2002
NGRDI	$(R_G - R_R)$ / $(R_G + R_R)$	Tucker 1979
$NDVI_{NIR, RED}$	$(R_{NIR} - R_R)$ / $(R_{NIR} + R_R)$	Rouse et al. 1974
$NDVI_{800,750}$	$(R_{800} - R_{750})$ / $(R_{800} + R_{750})$	Gnyp et al. 2015
$SR_{810,750}$	R_{810} / R_{750}	Gnyp et al. 2015

Table 1. Applied Vegetation indices. R = reflectance (%), R_R = red, R_G = green, R_B = blue, R_{NIR} = near-infrared, R_i =

Lussem, 2018 ISPRS - International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences

Low-cost assessment of vegetation indices

RGB im

- **Using FIJI to measure vegetation indices based on RGB indices. Manual calculation of the Normalized Green Red Difference Vegetation index (NGRDVI) based on the parameter Hue.**

1. Separate the three bands of the RGB image: Image -> Type -> RGB Stack and Image -> Stack -> Stacks to Images

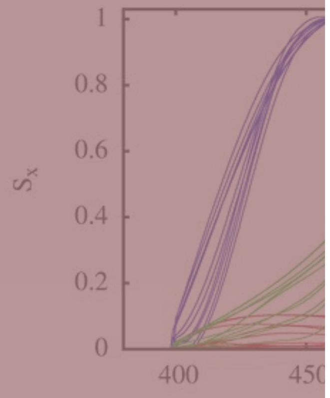
2. We can now do the math between the spectral "bands" of an RGB image. The calculation is:

$$NGRDVI = \frac{(Green - Red)}{(Green + Red)}$$

3. Process - > Image Calculator.

4. Anlyze -> Set Measurements -> “mean gray value"; Redirect to "None"; Decimal places "3”.

5. Analyze -> Measure



Typical spectral
cameras with R

Reference
Bendig et al. 2015
Louhaichi et al. 2001
Gitelson et al. 2002
Tucker 1979
Rouse et al. 1974
Gnyp et al. 2015
Gnyp et al. 2015

eflectance (%),
ar-infrared, R_i =

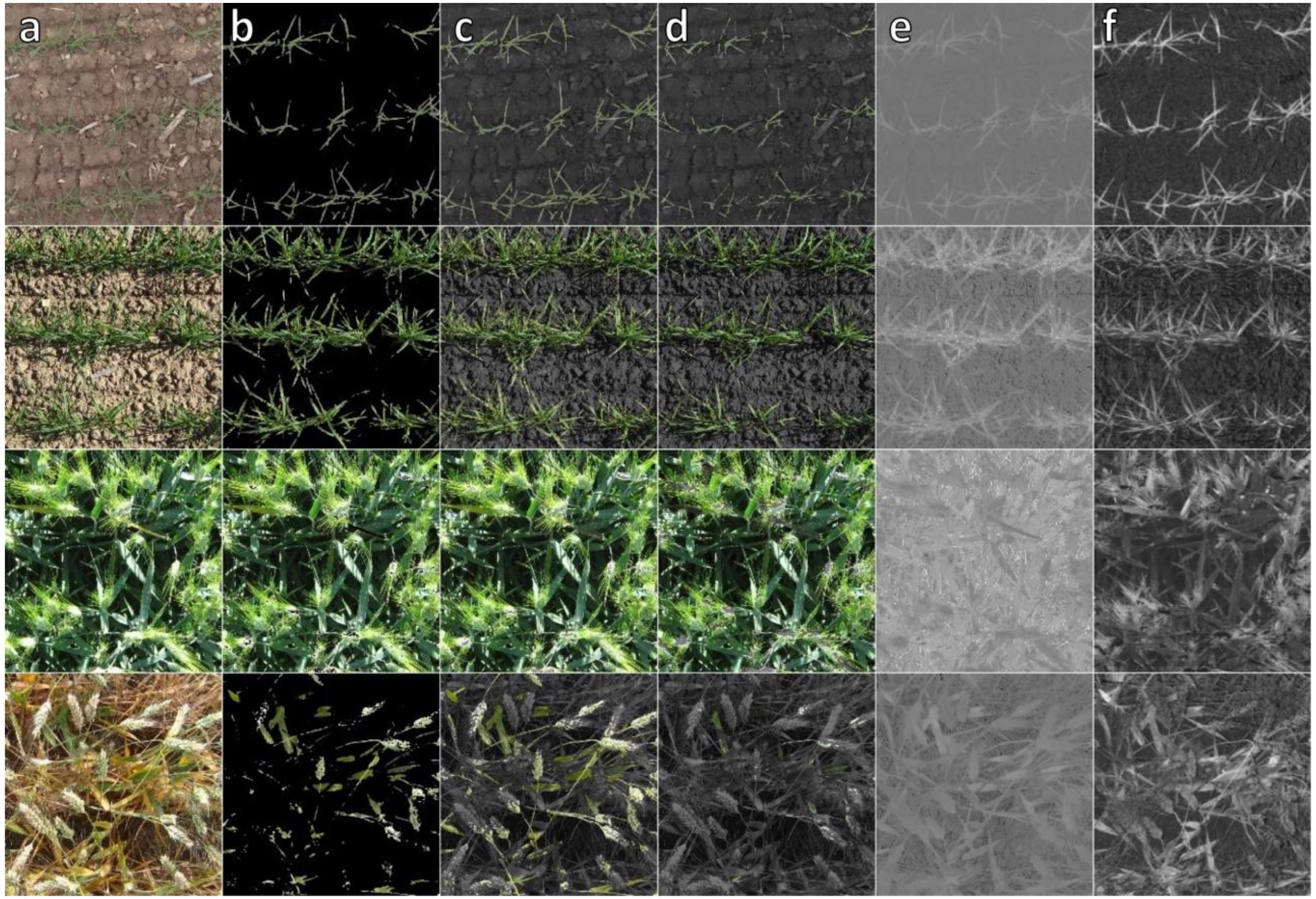
nal Archives of the Spatial Information Sciences

Low-cost assessment of vegetation indices

Fernandez-Gallego et al. 2019 *Eur. J. Agron.*

RGB imaging

Wheat experimental plots (INIA, Aranjuez 2017)

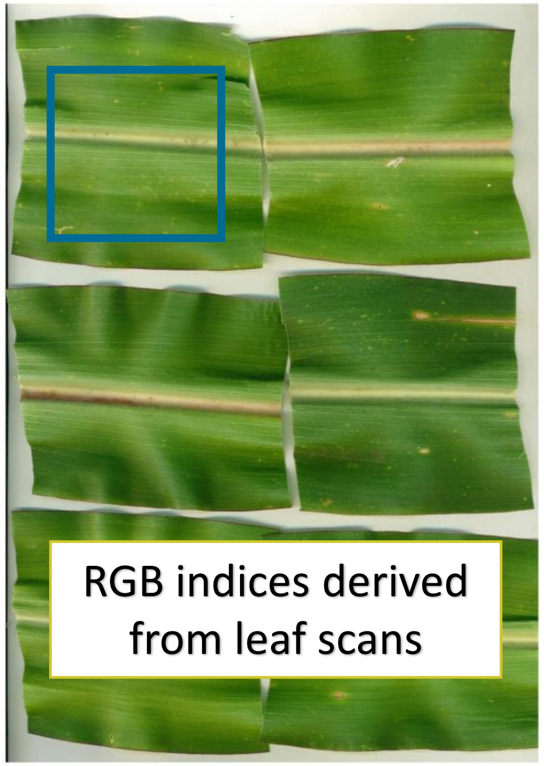


Images of wheat plots in different stages of growth: a) starting image and different vegetation indices evaluated: b) u'V'A; c) GA, d) GGA, e) NGRDI and f) TGI

Very ow sampling cost, high resolution and good repeatability

Platforms and image resolution

Aerial level RGB images



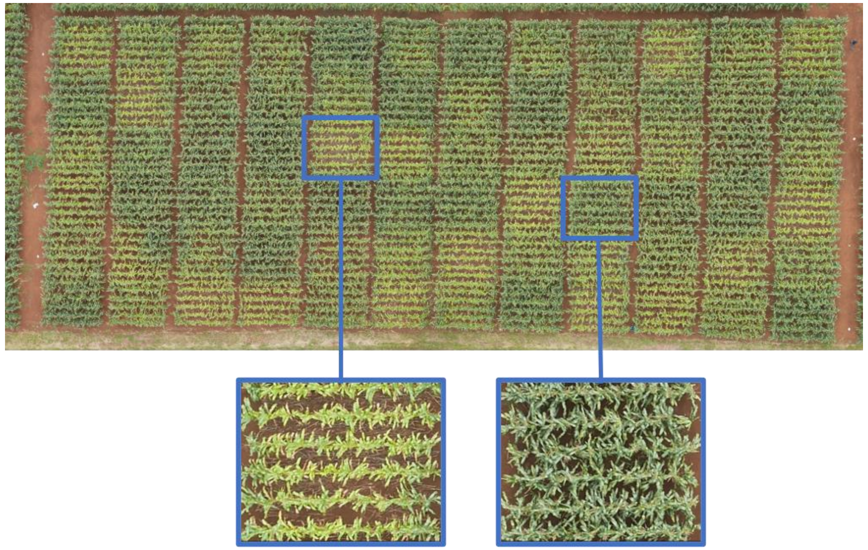


Image resolution

Gracia-Romero, 2017 *Front. Plant Scie.*

Platforms and image resolution

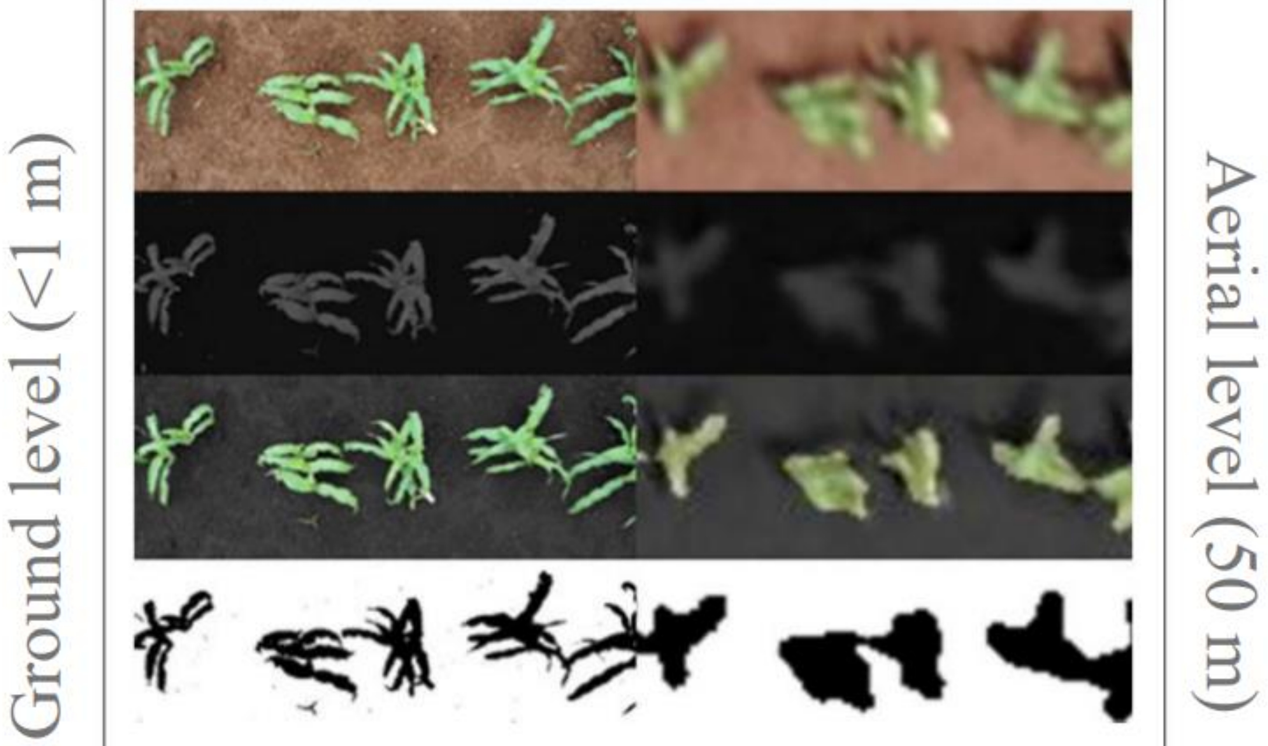
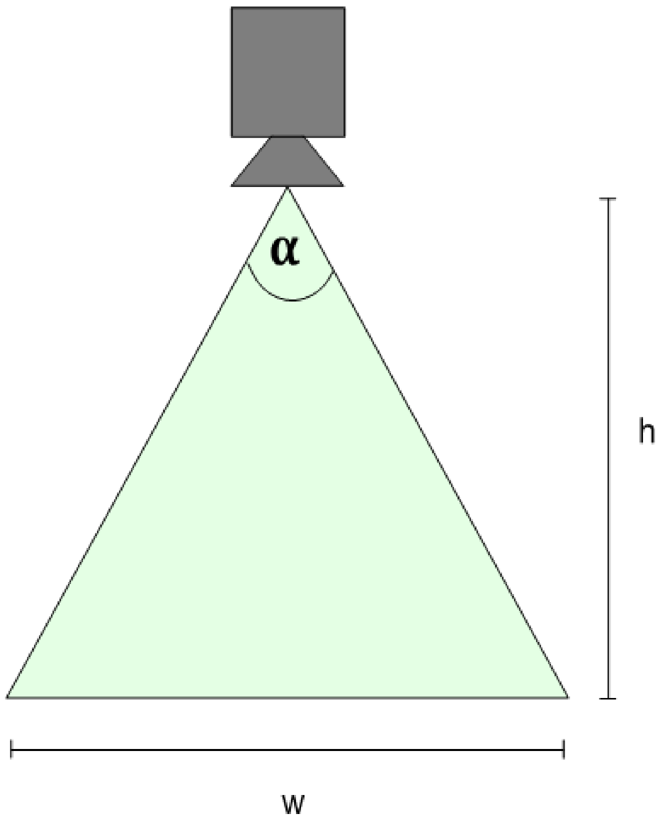


FIGURE 2 | Examples of the differences in resolution between images taken at ground level and aerially.

Calculation of VIs through conventional RGB cameras

Platforms and image resolution



Tau Core:	640	
Lens:	19	mm
Distance to ground (h):	50	m
Opening angle horizontal (α_h):	32	°
Opening angle vertical (α_v):	26	°
Resolution horizontal:	640	px
Resolution vertical:	512	px
Picture width on ground (w_h):	28.67	m
Picture heigth on ground (w_v):	23.09	m
Pixel per meter horizontal:	22.3	px/m
Meter per pixel horizontal:	0.045	m/px

Are drones becoming more accessible?

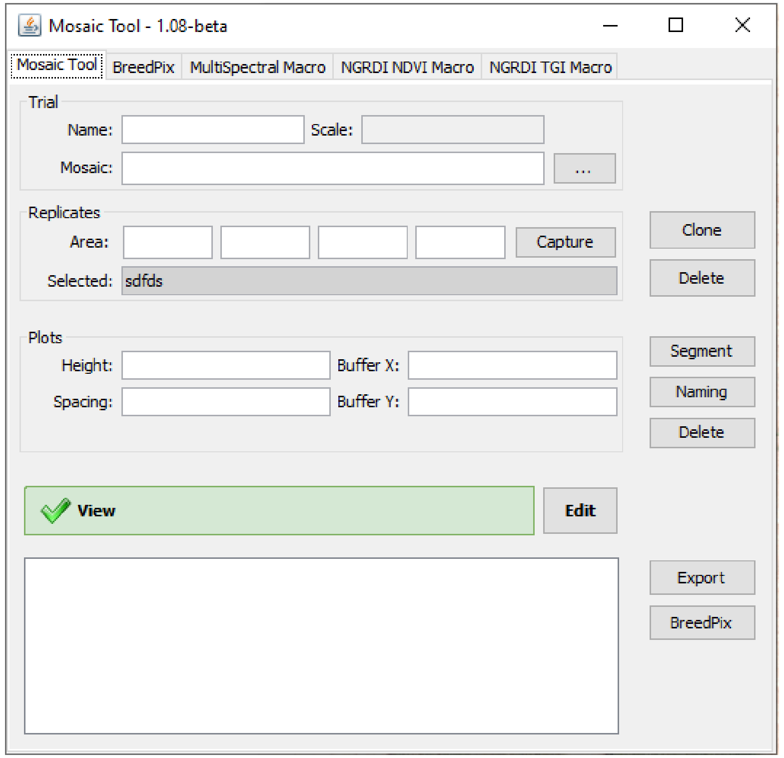
Drones are increasingly becoming a ubiquitous feature of society. […]This has been possible due, largely, to **significant developments in computerization** and **miniaturization**, which have culminated in **safer**, **cheaper**, **lighter**, and thus **<u>more accessible drones for social scientists</u>**.

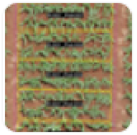
DJI Mavic

https://www.droneregulations.info/index.html

Low-cost assessment of vegetation indices

Automated RGB-Vis calculaton





MosaicTool

Project ID: 3184730

Uav Crop Phenotyping · Multispectral · Rgb · + 2 more

28 Commits · 1 Branch · 0 Tags · 10.6 MB Files · 10.6 MB Storage

Data extraction and processing workflow for RGB, VNIR and TIR UAV crop phenotyping.

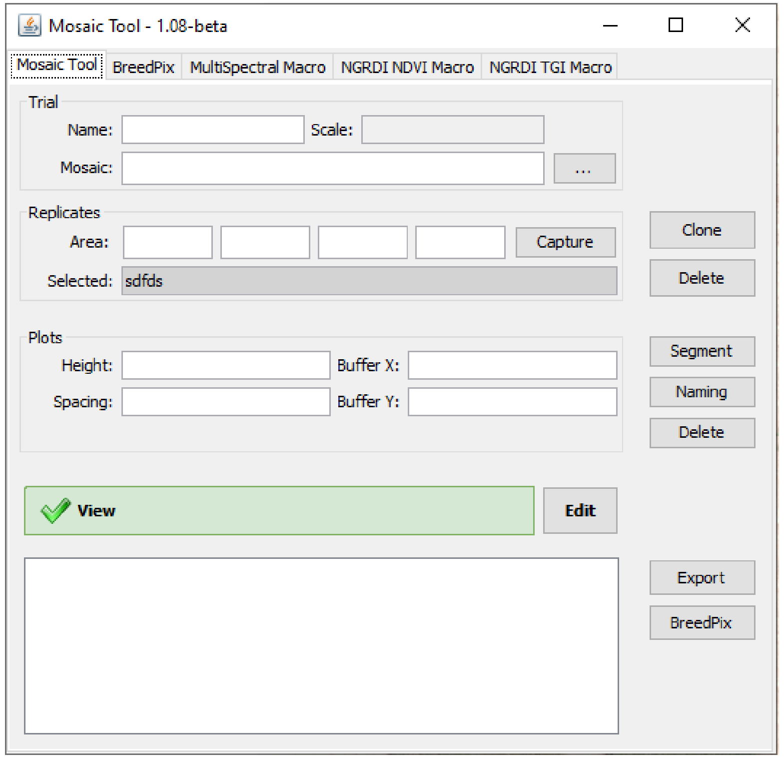
Authors

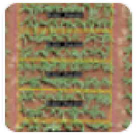
- Dr. Shawn Kefauver
  - Project Principal Investigator, University of Barcelona
- Adrian Gracia-Romero, University of Barcelona
- Prof. José Luis Araus, University of Barcelona
- George El-Haddad
  - Software Engineer, Postlight

Scan the QR to get more info and download "MosaicTool"

Low-cost assessment of vegetation indices

Automated RGB-Vis calculaton





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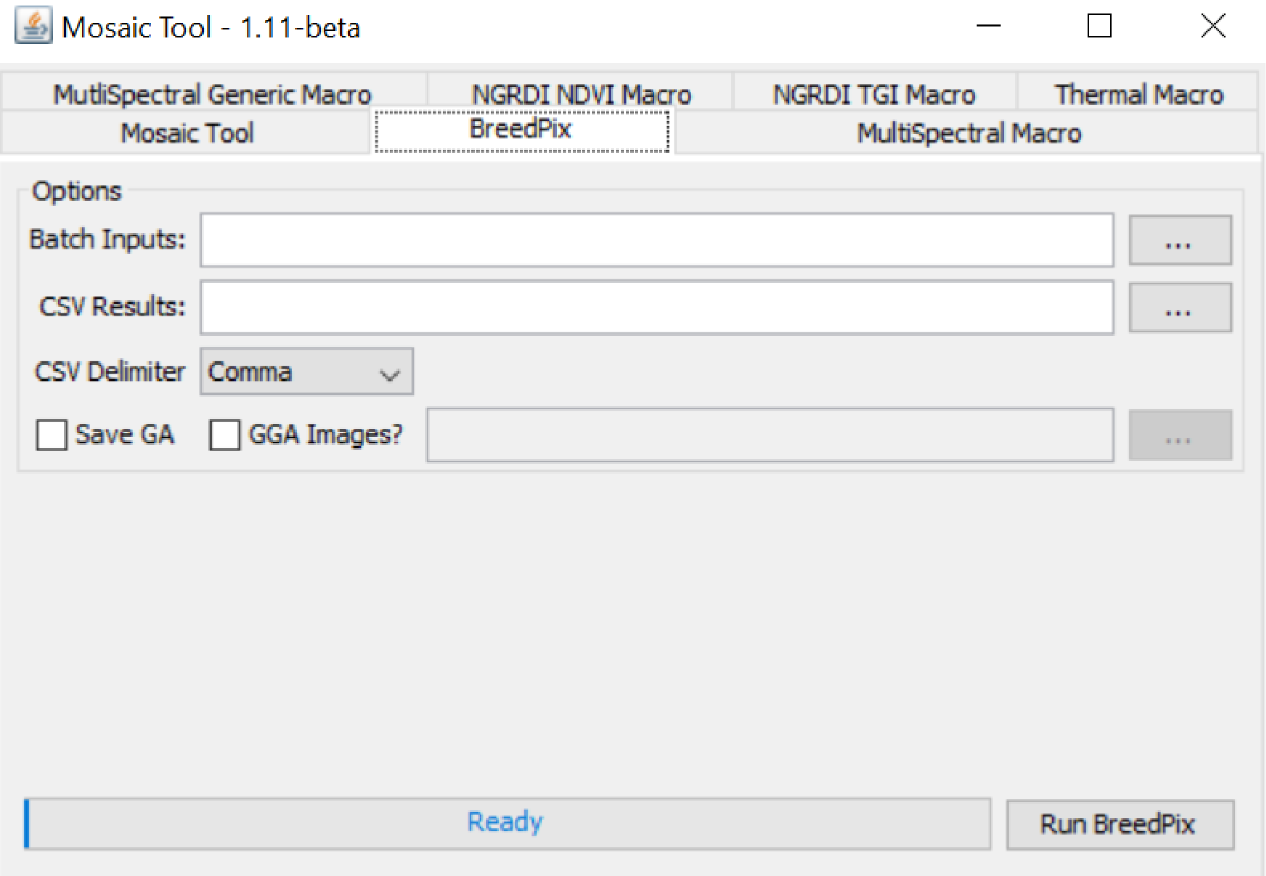
Automated RGB-Vis calculaton

Image processing tab shows various processing options to enable on the batch selection of images



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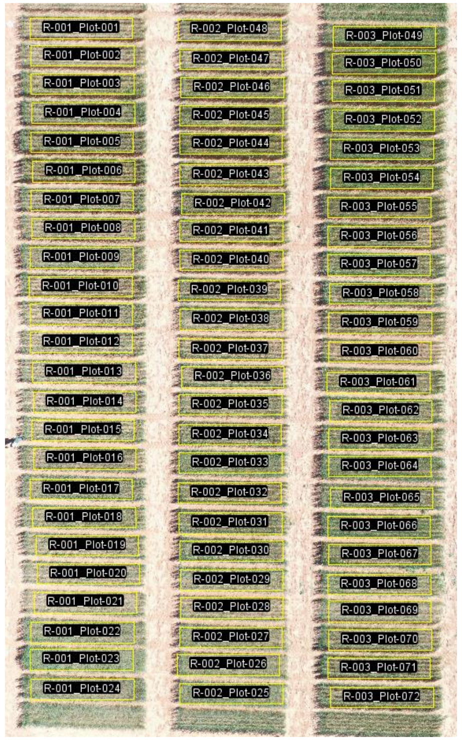
Low-cost assessment of vegetation indices

Automated RGB-Vis calculaton

Semi-automatic segmentation of aerial images and to formulate VIs for both RGB, multispectral and thermal images



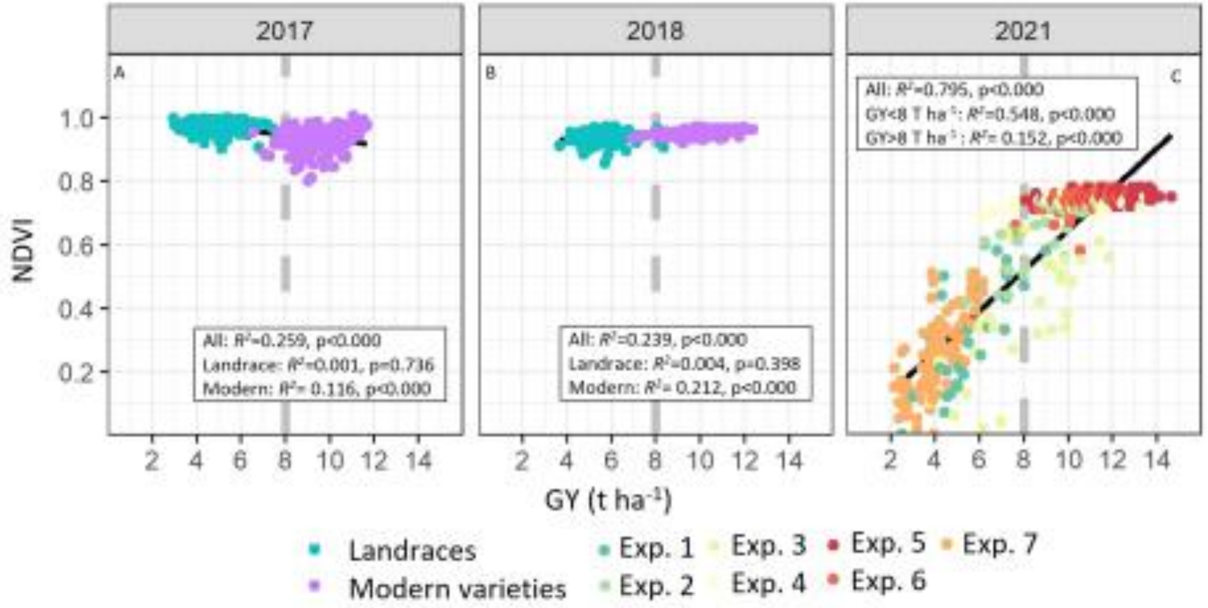
Scan the QR to get more info and download “MosaicTool”

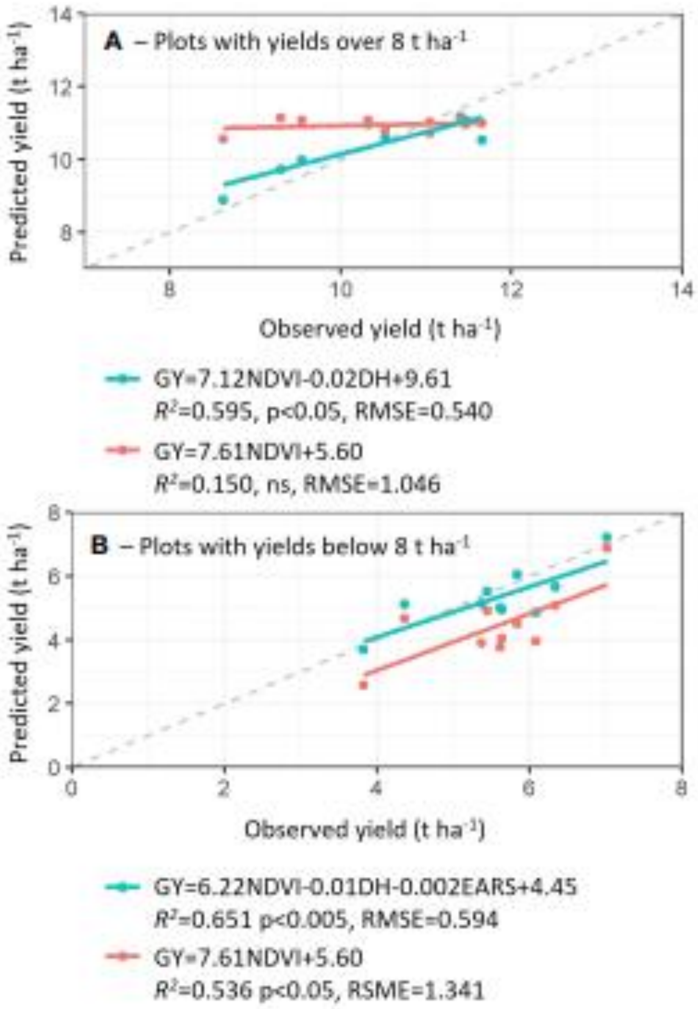
Calculation of VIs through conventional RGB cameras

Gracia-Romero, 2023 *Front. Plant Scie.*

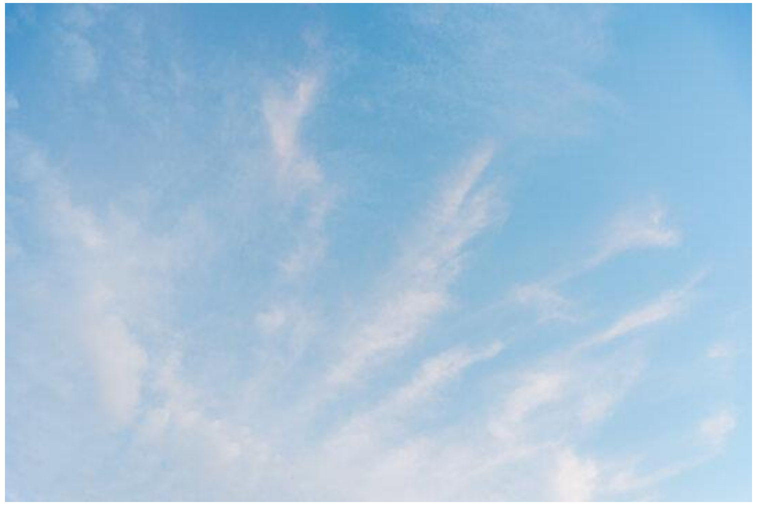
Limitations

As other VI, they get saturated at high LAI. (e.g. determined genetic material as **landraces** or modern varieties at stages with much green biomass, under **high yielding conditions**)





Limitations



Ideal conditions

Clear, sunny weather with minimal cloud cover in the mid-morning or mid-afternoon when the sun is at a moderate angle and the light is even.

Possible source of error

Shadows and fluctuating ambient lighting

Best to avoid taking images during periods of high wind or rain, as this can cause blurring and distortion in the images.

Take home messages

- Very low sampling cost and high resolution.
- Good repeatability.
- Validated as vegetation indices.
- Calculation of RGB-VIs can be automated.

Thank you for your attention

Integrative Ecophysiology group

Sustainable Field Crops
Efficient Use of Water in Agriculture

Generalitat de Catalunya
Government of Catalonia

IRTA

Institute
of Agrifood Research
and Technology

RGB low-cost imaging for plant phenotyping: index calculation and analysis

Training programme on Affordable Phenotyping
Matera-Metaponto, Italy 18-21 April 2023

EMPHASIS

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