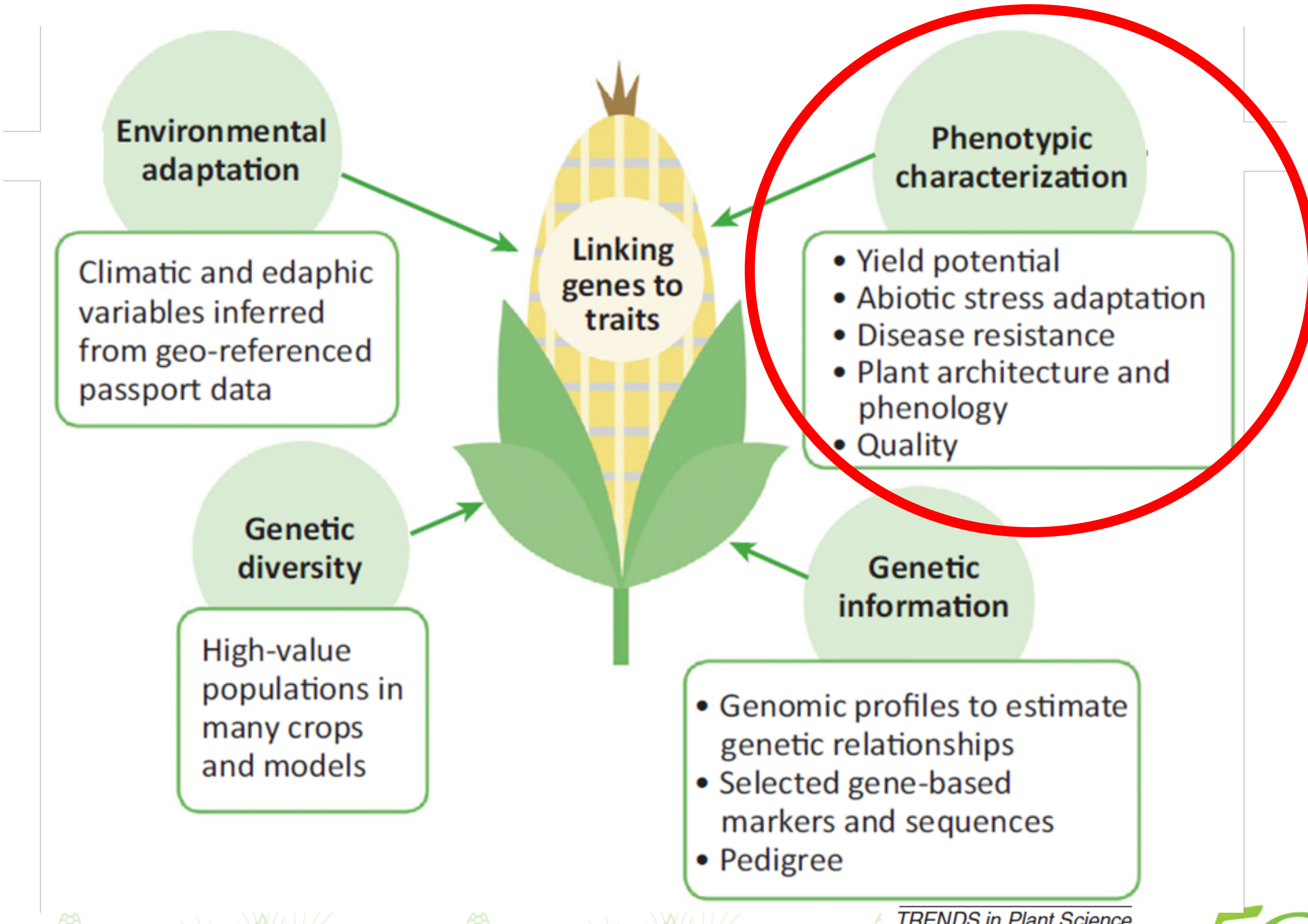
High-throughput phenotyping in maize breeding

The CIMMYT's experience

Crop breeding pillars

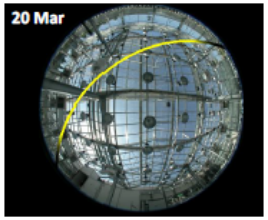


Araus and Cairns 2014

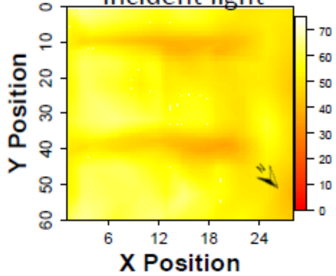
Phenotyping – still a bottleneck

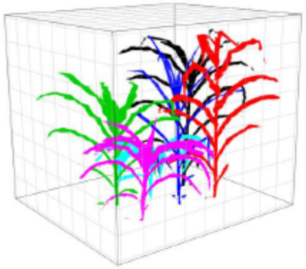
Controlled Environments

Daily sun paths

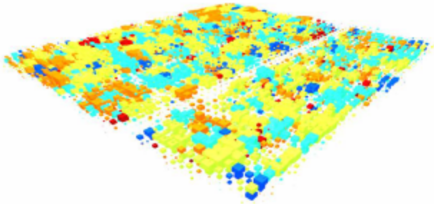


Spatial distribution of incident light





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.

1966-2016 CIMMYT

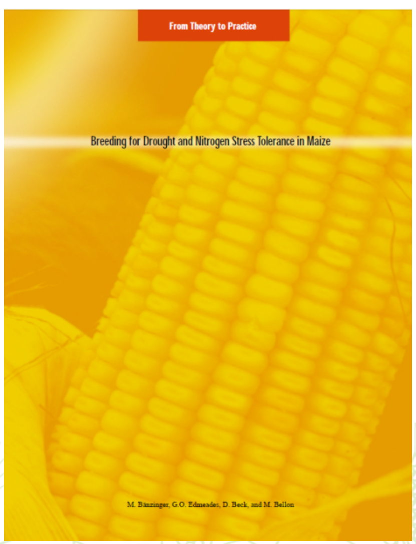
Traits

anthesis-silking interval

Bolaños and Edmeades 1996 FCR

Tools

Manuals



Banzinger *et al. 2000*

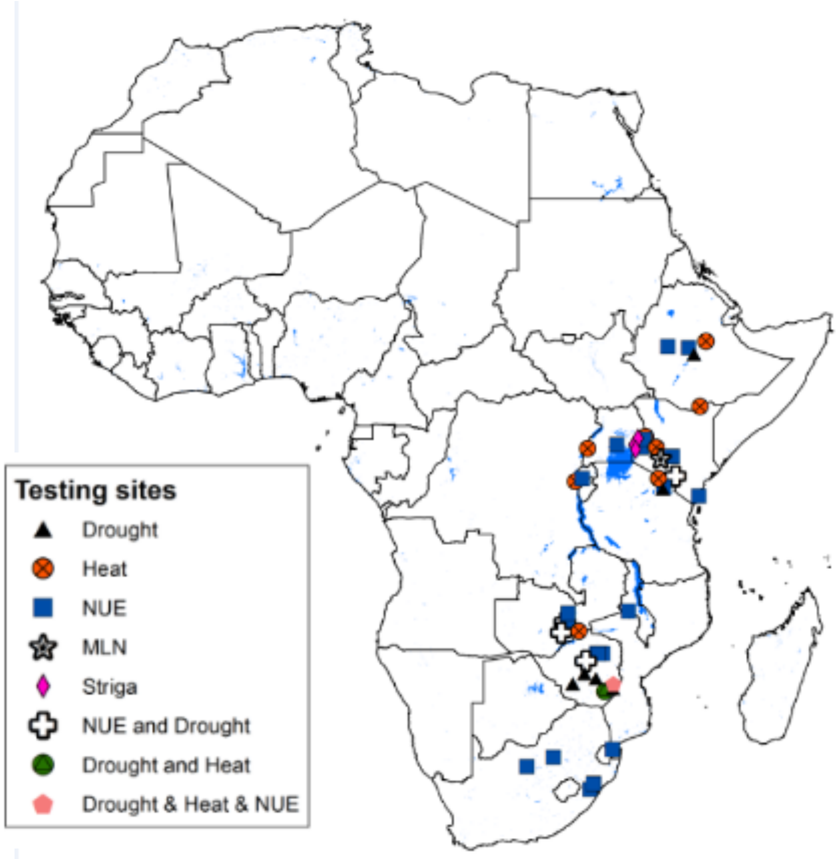
Iván Ortiz-Monasterio

1966-2016 CIMMYT

Outline

- Field phenotyping: multilocation – facilities
- Proximal sensing & imaging: affordable alternatives
  - RGB – indices
  - Other uses of RGB images
  - Lab approaches: NIRS
- More than just phenotyping
- Conclusion

Large testing network



Cairns updated from Prasanna et al. 2013

Drought* - 61 ha

Low nitrogen - 48.5 ha

Heat - 13.5 ha

MLN - 17 ha

*Including CFT sites

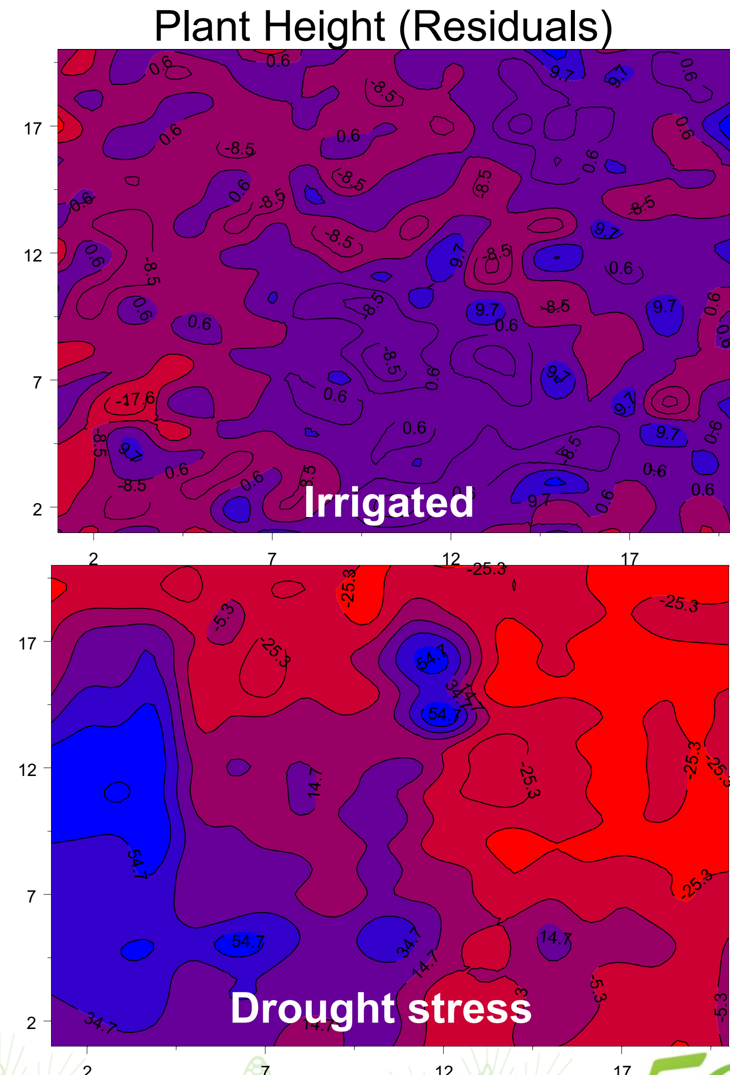
Reducing field variability: managed growth conditions

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.*

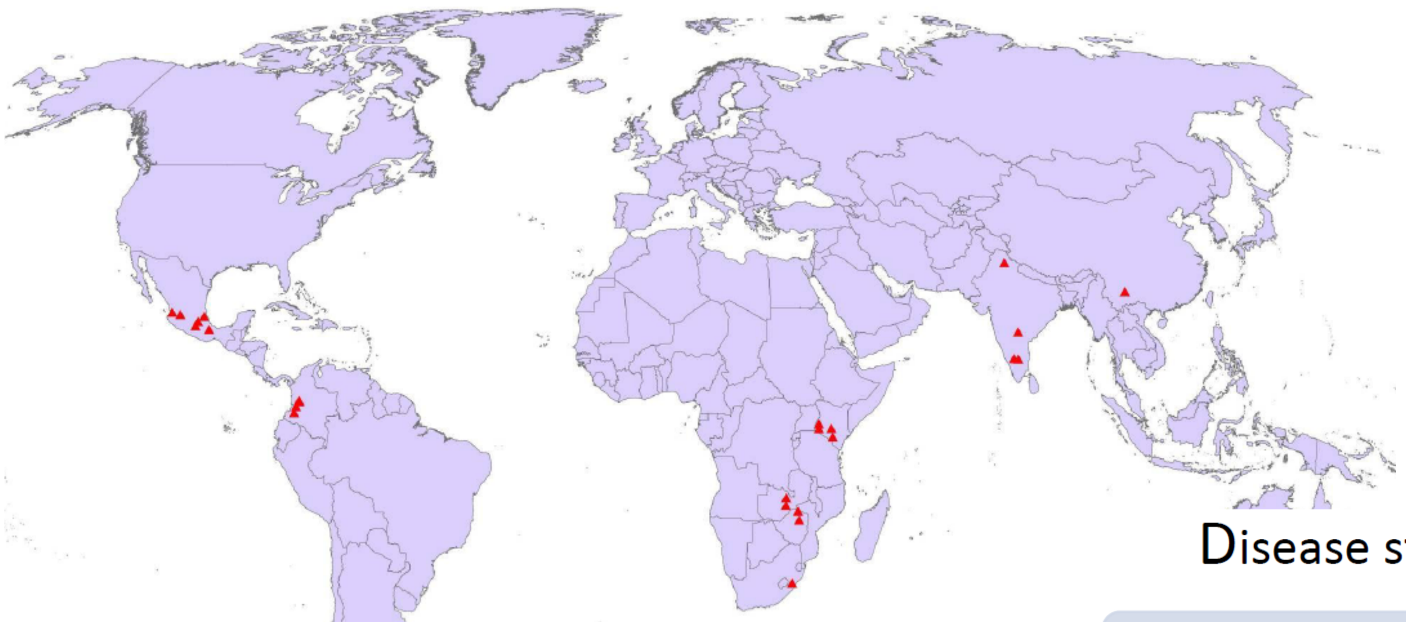
Test environment	Variance components‡		
	σ^2_g	σ^2_{ge}	σ^2_ε
Early maturity group			
Optimal	28.02 ± 11.14	24.17 ± 8.24	47.81 ± 13.95
Managed drought	14.39 ± 9.30	14.58 ± 4.17	71.04 ± 8.08
Random abiotic stress	10.29 ± 8.32	23.37 ± 11.76	66.34 ± 14.75
Low N	19.01 ± 10.66	23.86 ± 11.30	57.13 ± 14.18
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.*



Burgueño and Wilcox

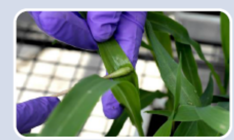
Disease phenotyping sites



Disease stress imposition

Natural infestation

Hotspot



Artificial infestation

Inoculation

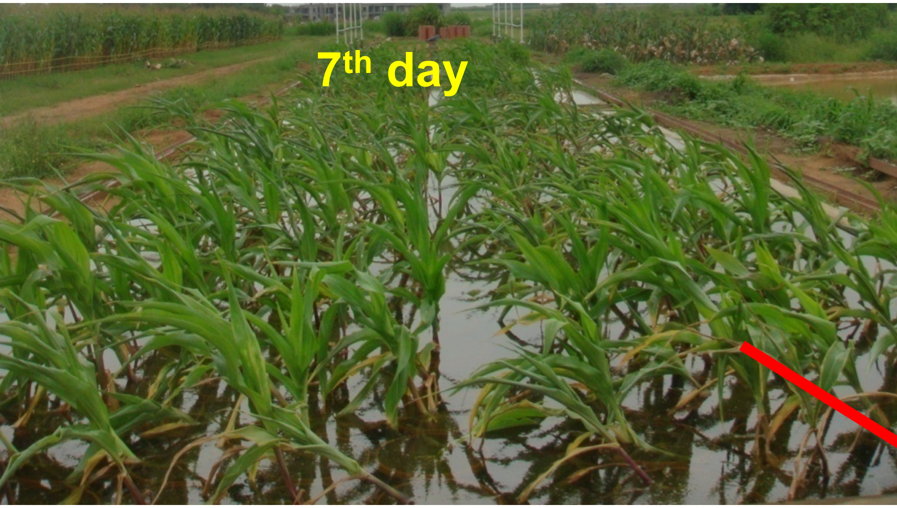
Facilitated infestation

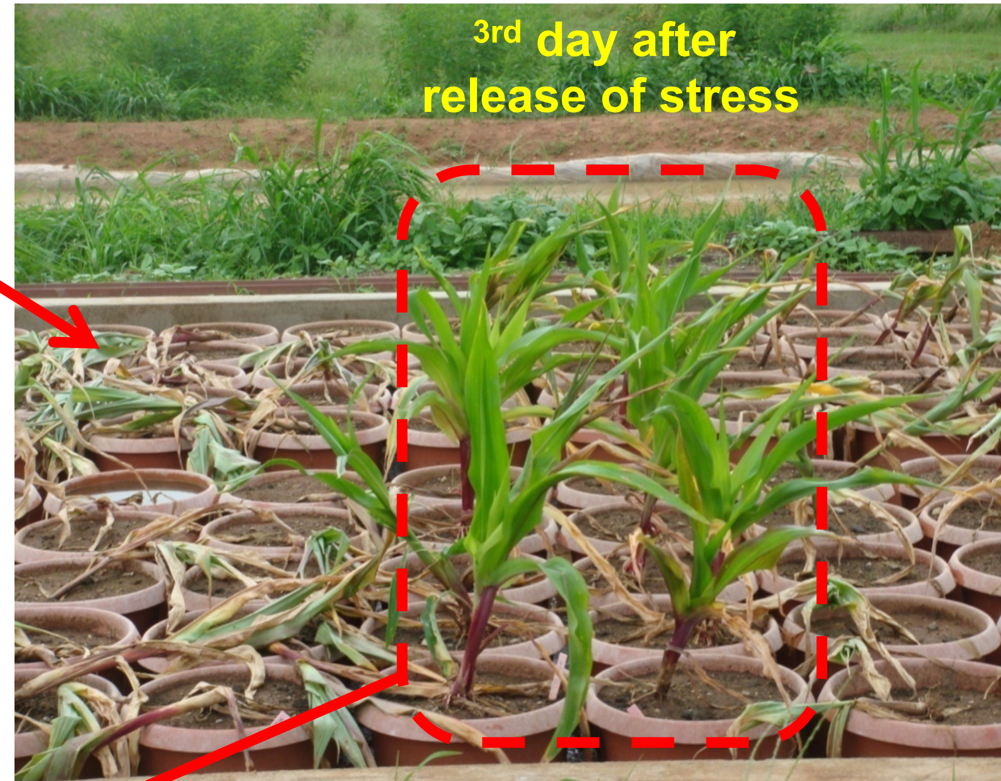
Misting

Adapted from Cairns

1966-2016
CIMMYT

Water-logging at vegetative growth stage





Adapted from Zaidi

Root phenotyping

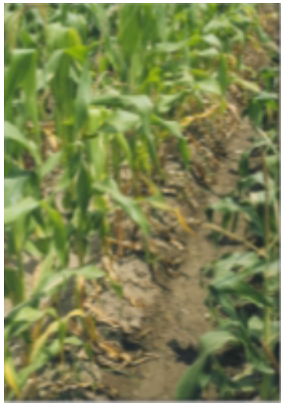
- ***Structural traits:*** ***root depth, length , volume, root-length density, dry weight***
- ***Functional traits:*** ***water use during stress (WU) & Transpiration efficiency (TE)***

Adapted from Zaidi

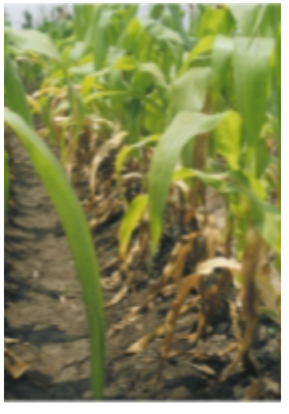
Visual scores: e.g. canopy senescence

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%)

Banzinguer *et al.* 2000

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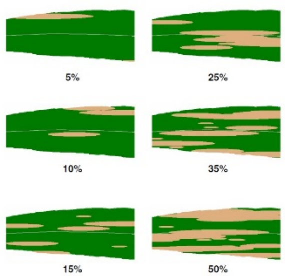
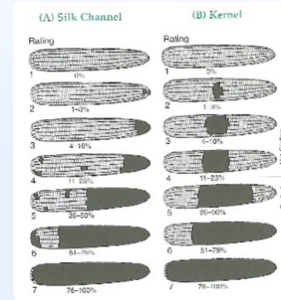
Visual scores



E. turcicum blight



Ear rots by different inoculation methods

Disease scoring

FRS

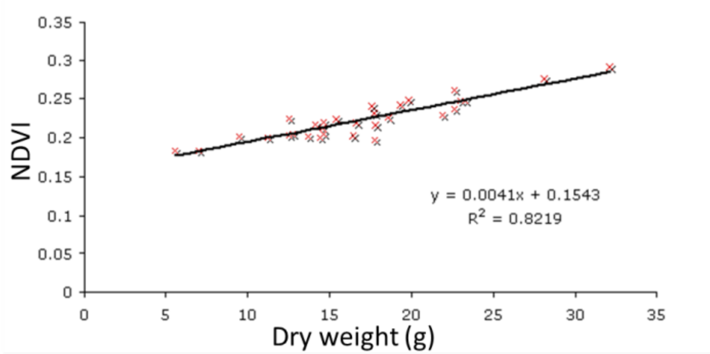
Ear rot resistance

Standard area diagrams and scales

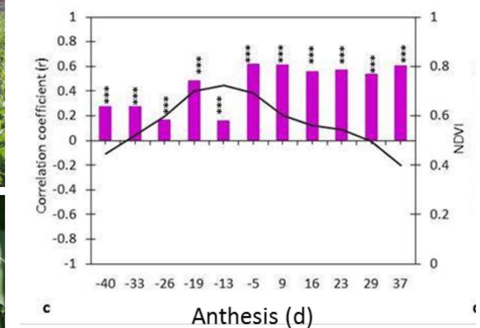
Cairns, adapted from Mahuku and Ngugi

Phenotyping tools: remote sensing approaches

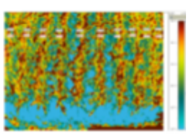
Biomass

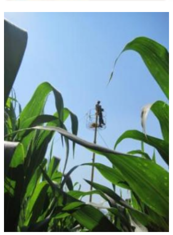


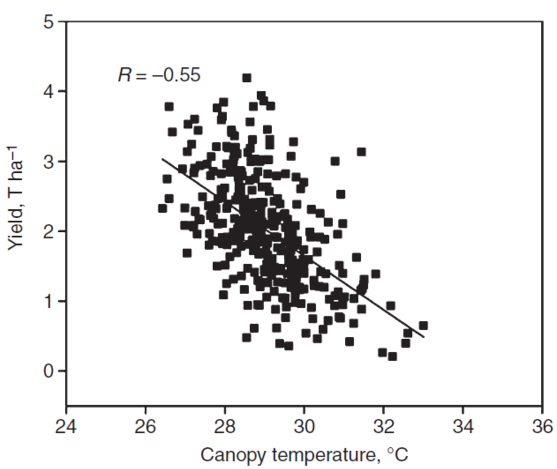
Senescence



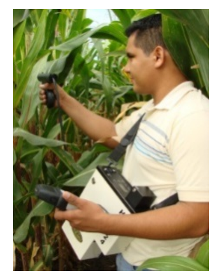
Plant water status

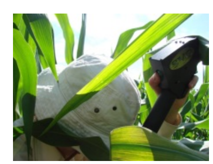


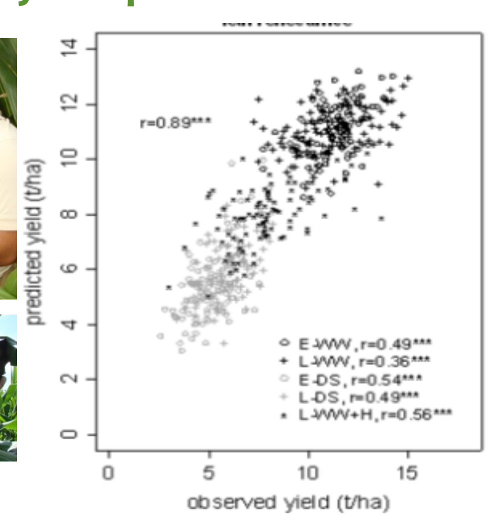




Grain yield prediction

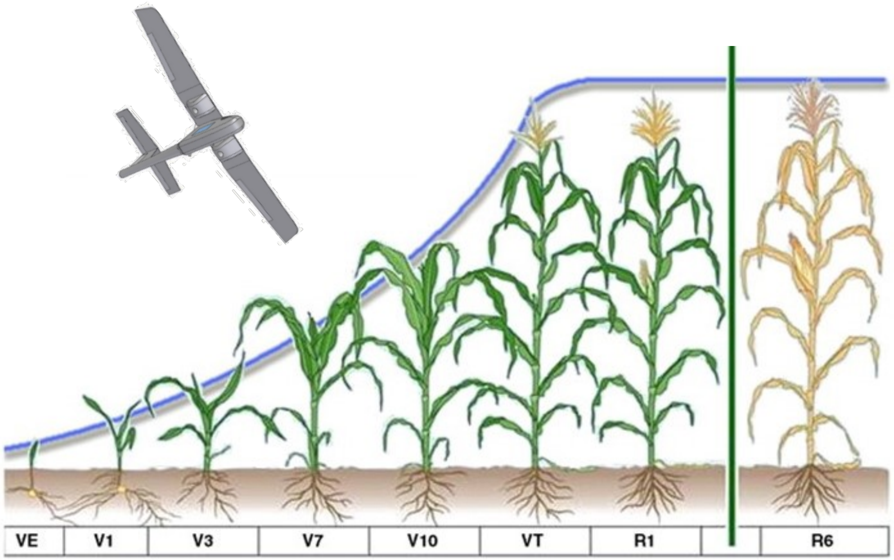


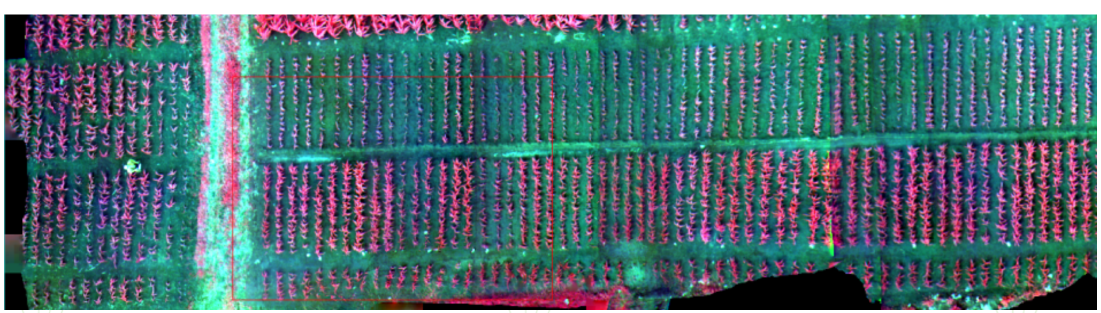




Masuka *et al.* 2012; Romano *et al.* 2012; Weber *et al.* 2012; Zia *et al.* 2013; Cairns *et al.* 2013, Zaman-Allah *et al.* 2015, Vergara *et al.* 2016

Aerial platforms





Zaman- Allah *et al.* 2015 Plant Methods

Kefauver et al. 2016 Harare in preparation Harare

1966-2016
CIMMYT

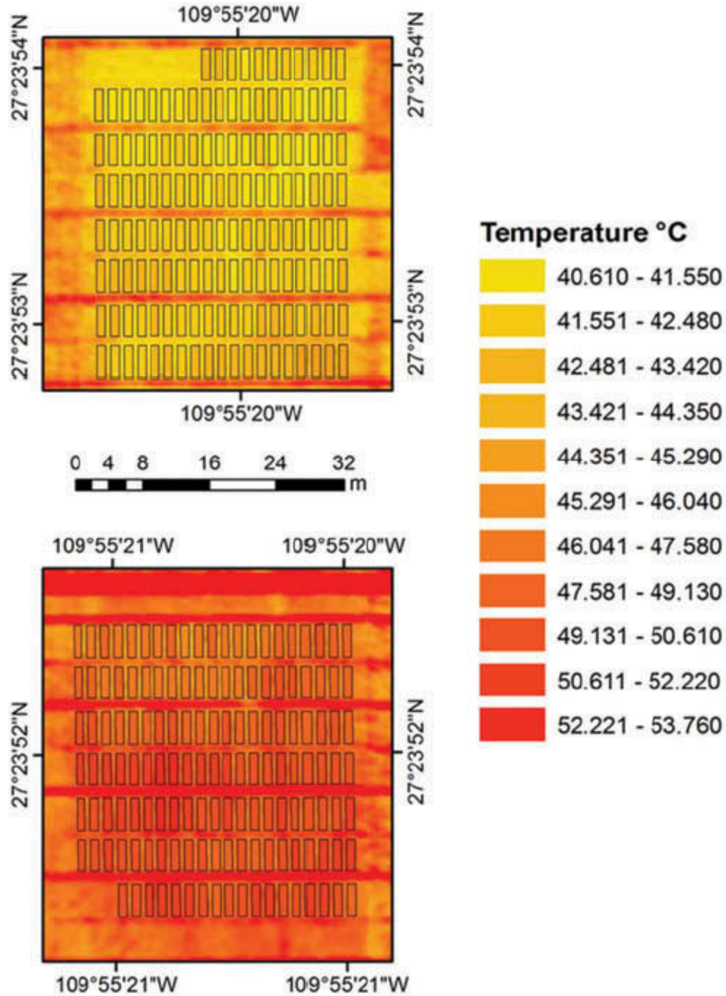


TABLE 5 Expected genetic gains for indices S1 (grain yield, canopy temperature, NDVI, anthesis silking interval), S2 (anthesis silking interval, canopy temperature, and NDVI) and S3 (canopy temperature and NDVI) on grain yield (GY) under heat (HS) and combined heat and drought stress (HS + DS)

Index	GY
HS	
S1	0.486
S2	0.322
S3	0.237
HS+DS	
S1	0.015
S2	0.002
S3	−0.027

Neiff et al. 2015. J. Crop. Improv.

Affordable methods for biotic and abiotic stress phenotyping

Data collection

Present: Visual scoring

Score = 8
5% leaf loss

Score = 5
35% leaf loss

Score = 2
80% leaf loss

Future: Canopy digital imaging

Adapted from Cairns

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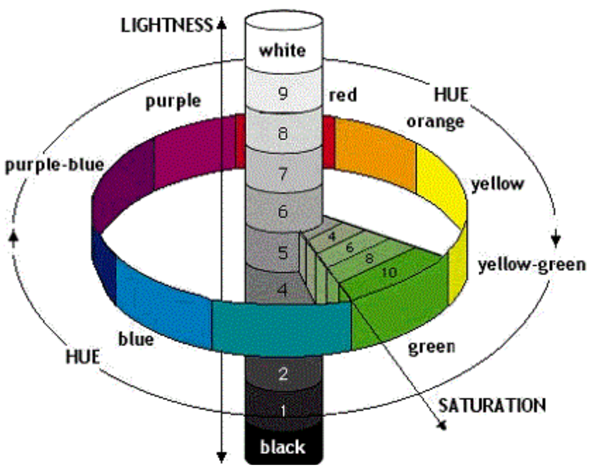
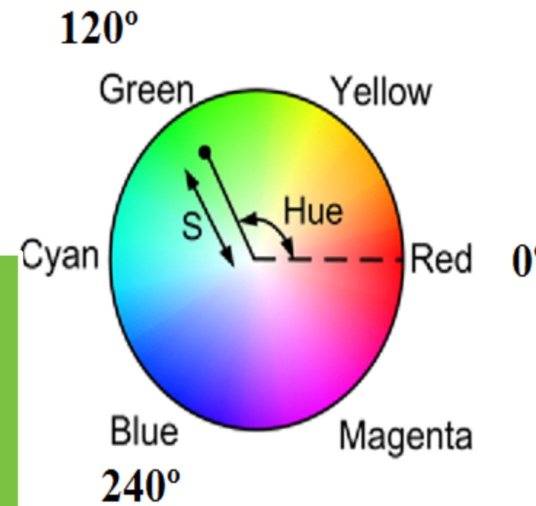
CIMMYT

RGB – indices



HIS color space

Hue



Green Area (GA)
(% pixels with 60º < Hue < 120º)

Greener Area (GAA)
(% pixels with 80º < Hue < 120º)

RGB, Green Area, Greener Green Area

MLN plot score 3.0

Maize Leaf Plot RGB

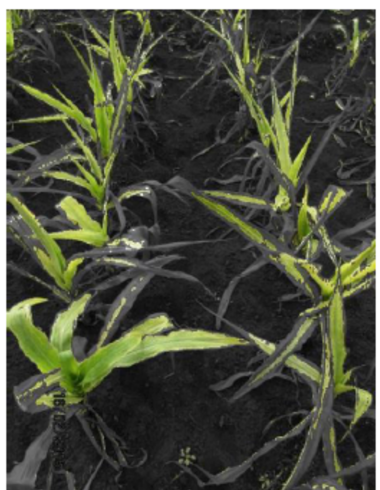
GA (healthy pixels)

GGA (very healthy pixels)

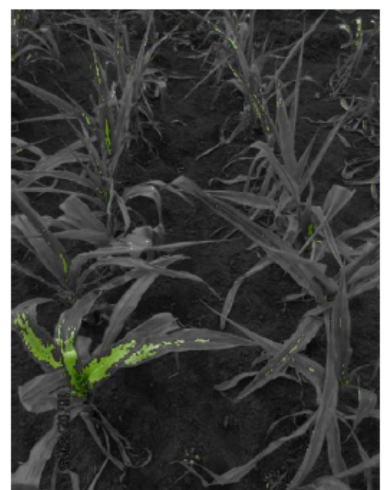
NGRDI (vigor index)

MLN plot score 4.0

Maize Leaf Plot RGB



GA (healthy pixels)



GGA (very healthy pixels)

NGRDI (vigor index)

Kefauver *et al.*

1966-2016 CIMMYT

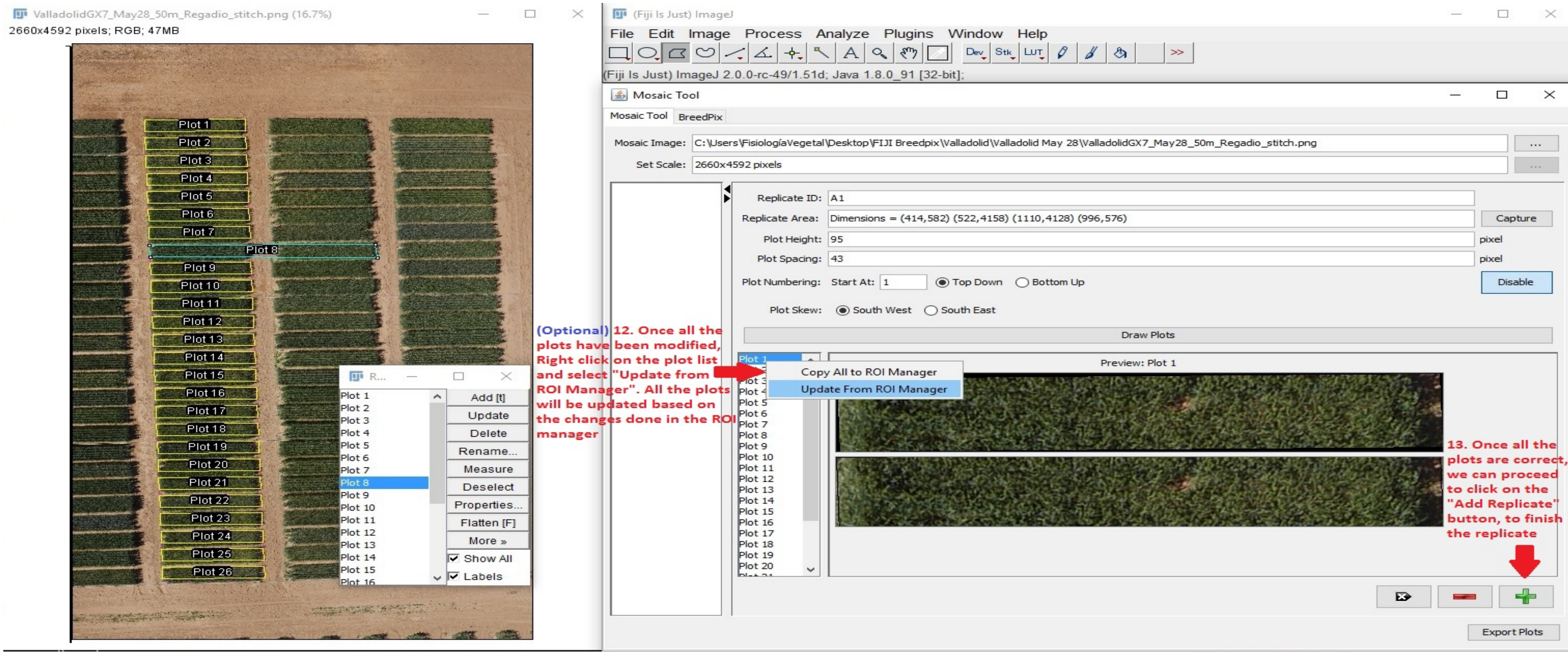
RGB image processing: vegetation indices

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 image processing: image segmentation

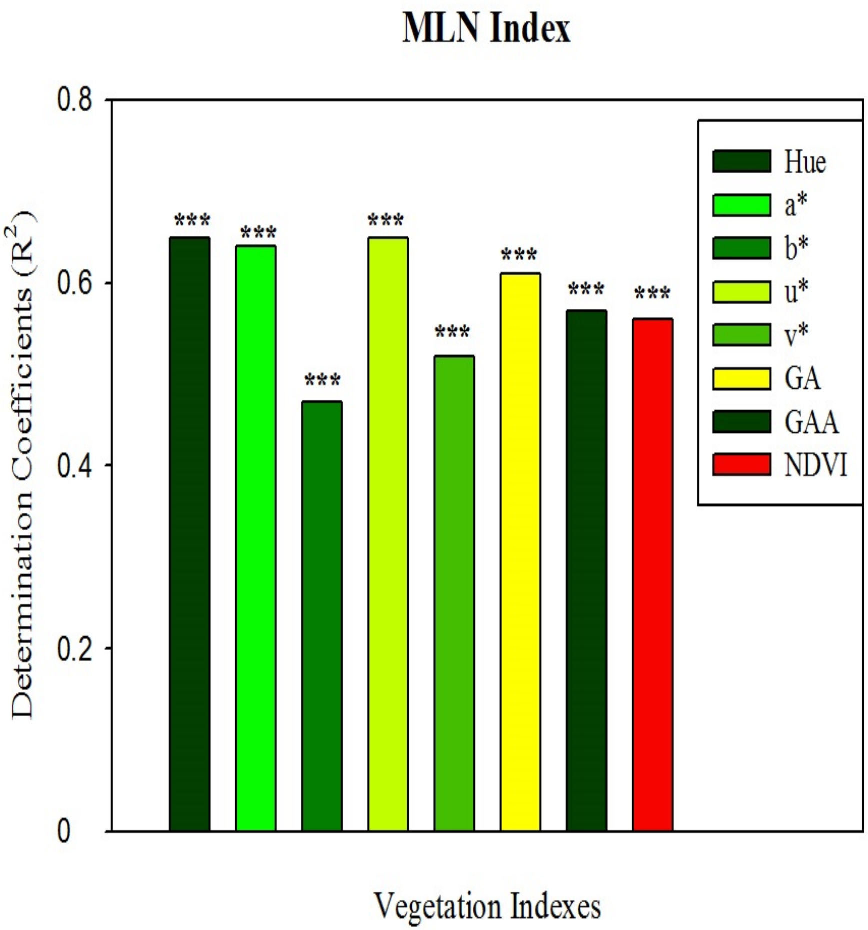


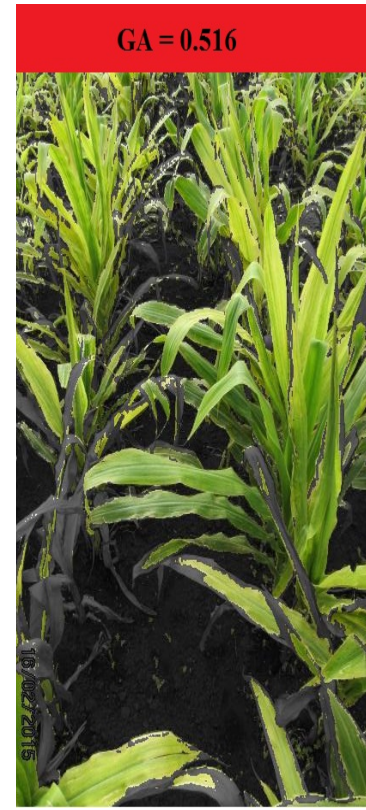
Kefauver *et al.*

1966-2016 CIMMYT

RGB vs Spectral indices

Maize Lethal Necrosis evaluation in Kenya





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

Kefauver *et al.* in preparation

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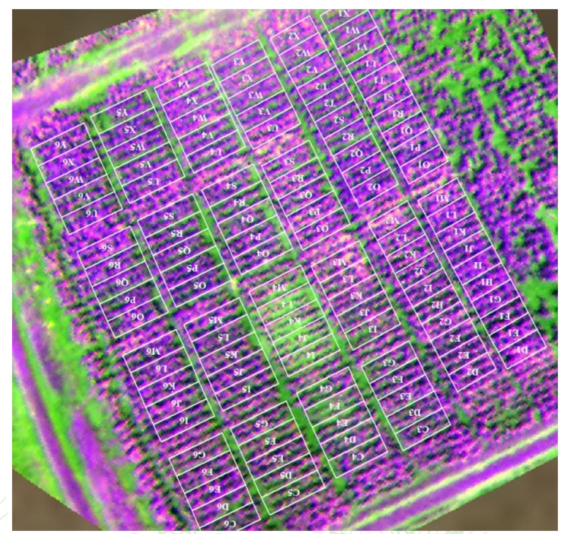
RGB vs Spect. indices

N fertilization treatments in Zimbabwe

CIMMYT's South Africa regional station, Harare



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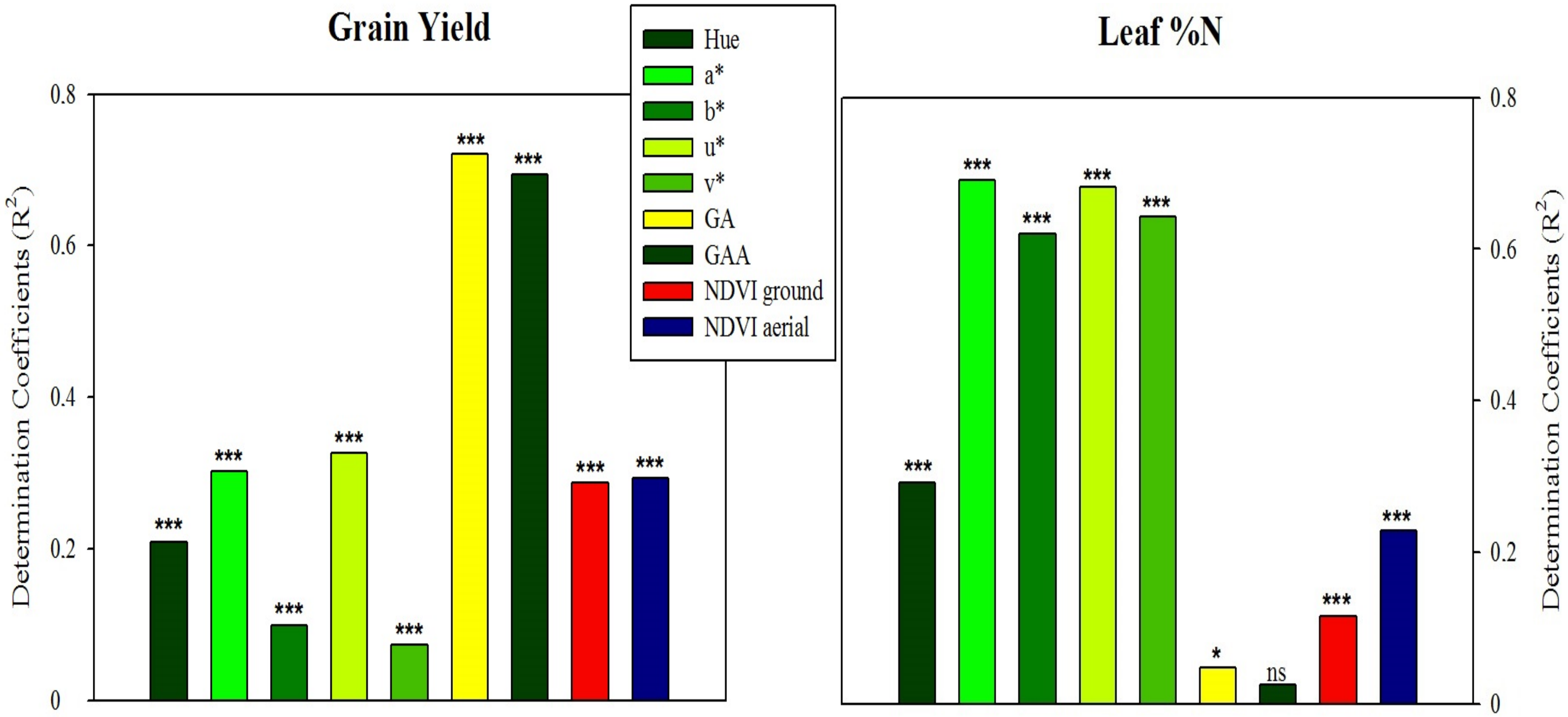
RGB vs Spectral indices

N fertilization treatments in Zimbabwe



Vegetation Indexes

Zaman-Allah et al. 2015. *Plant Methods*

Vergara et al. 2016 *Frontiers in Science*

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

Other applications of RGB images: harvest index

Kernels

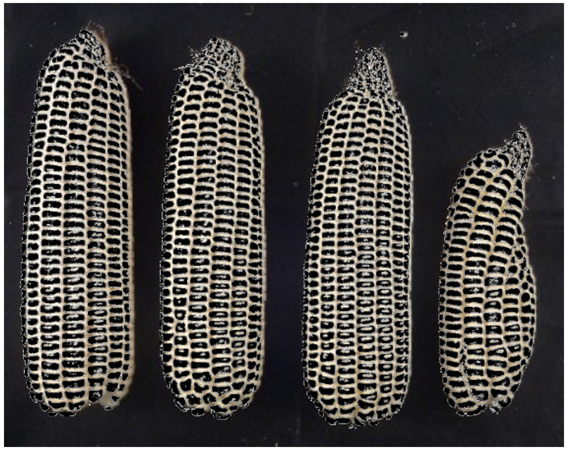
- Size
- Uniformity/abortion rate
- Kernel weight
- Rot index

Ears

- Number
- Rows
- Size

Stress stage

- Flowering stress
- Grain filling stress

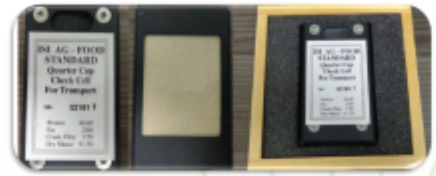


Zaman-Allah *et al.* *in preparation*

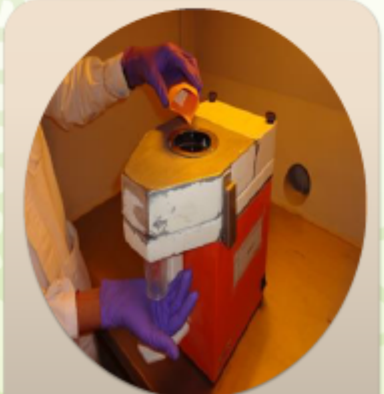
NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

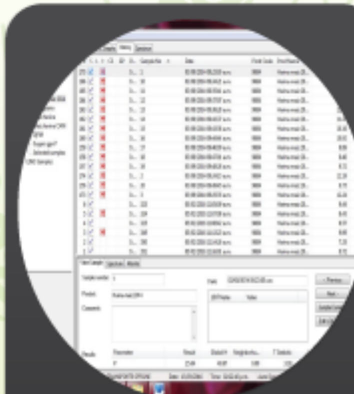
VERIFICATION

1. Advanced diagnostic
   - Wavelength linearization
   - Gain test
   - Self test
2. Routine diagnostics
   - Performance test
3. Check cell



SAMPLE ANALYSIS (Starch, protein, oil, provitamin A, lysine, tryptophan, anthocyanins





Total time: ~ 4 min

Adapted from Palacios-Rojas

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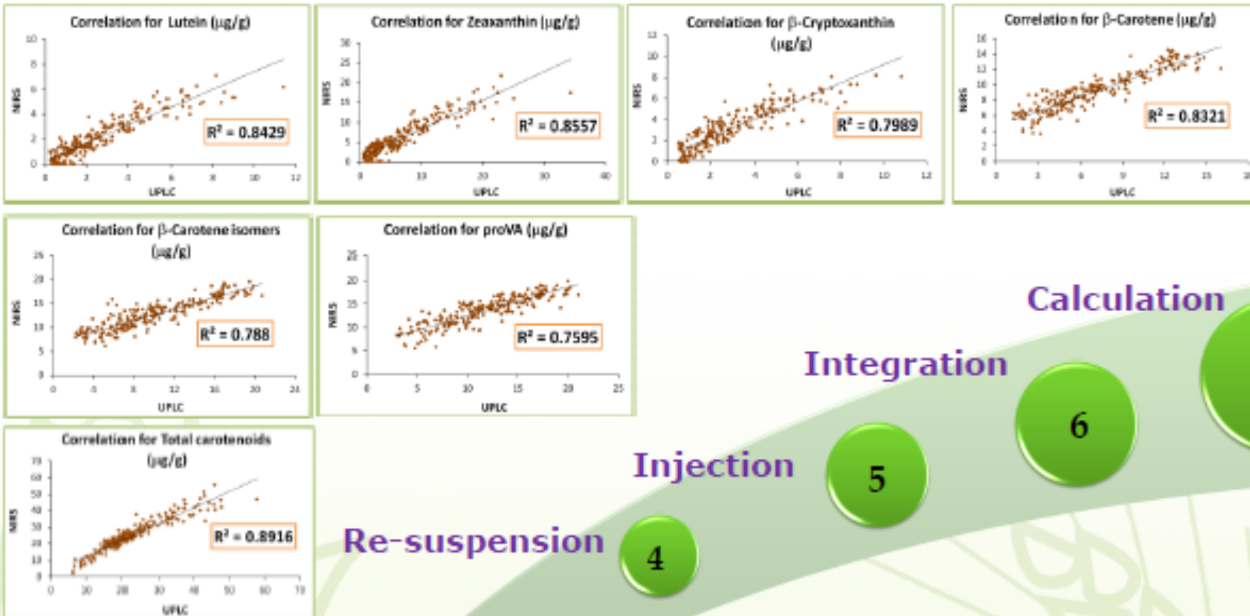
UPLC and NIR for carotenoids in maize



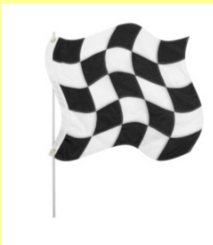
UPLC

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

NIRS as alternative to the analysis of other traits



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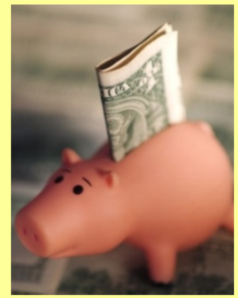


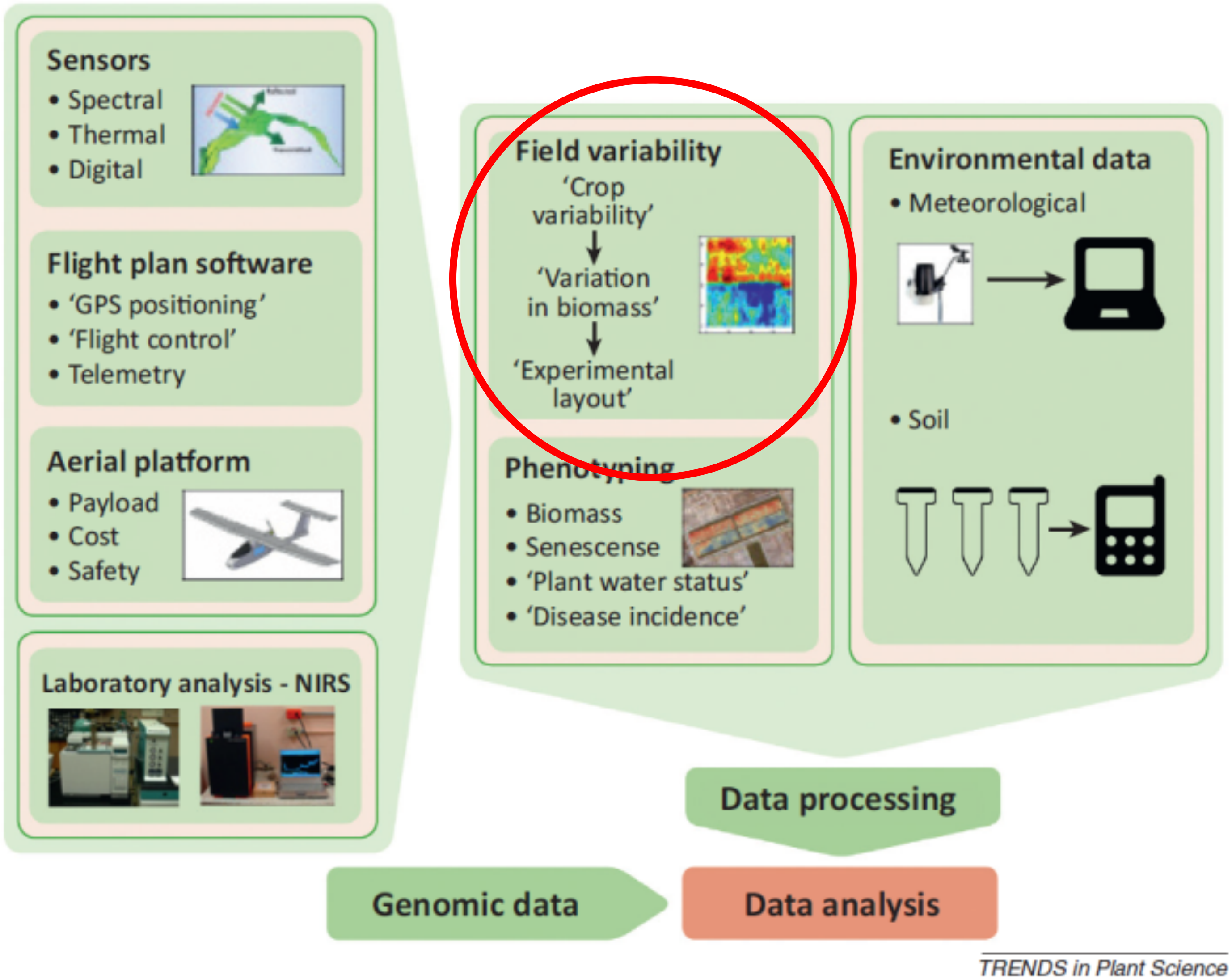
\+

Rapid + **Low technical skills** + **Cost-effective** + **Precise-enough**

Technique	IRMS		EA	AACC Method	NIRS-prediction			
Parameter	$\delta^{13}C$	$\delta^{18}O$	N content	Ash content	$\delta^{13}C$	$\delta^{15}N$	Ash	N
Cost per sample	10$	20$	3$	1.5$	1.0$			
Time	<10 min	<10 min	<10 min	≈24 h	≈3 min			
Equipment	EA-IRMS		EA	Muffle furnace	NIR spectrometer			

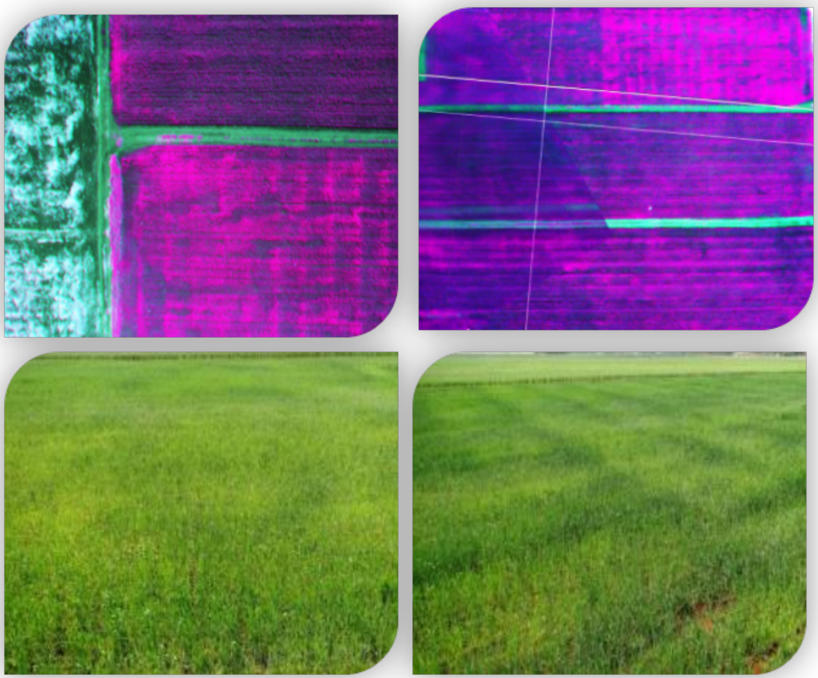
Cabrera-Bosquet *et al.* 2012 J. Agric. Chem.

More than traits and tools

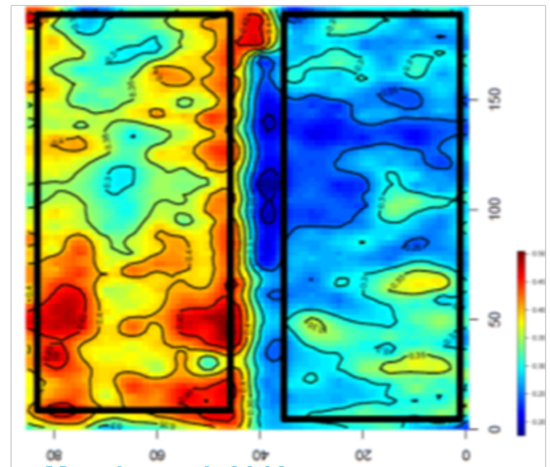


Araus and Cairns 2014

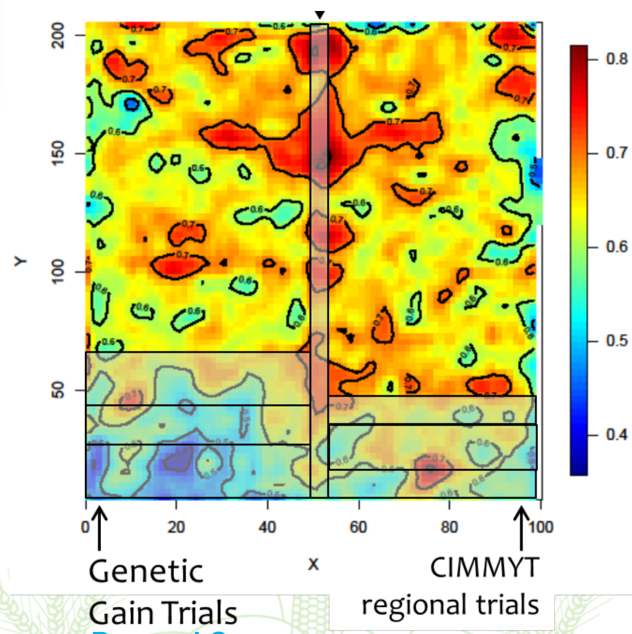
Measuring / reducing spatial variability



Cairns and Zaman-Allah

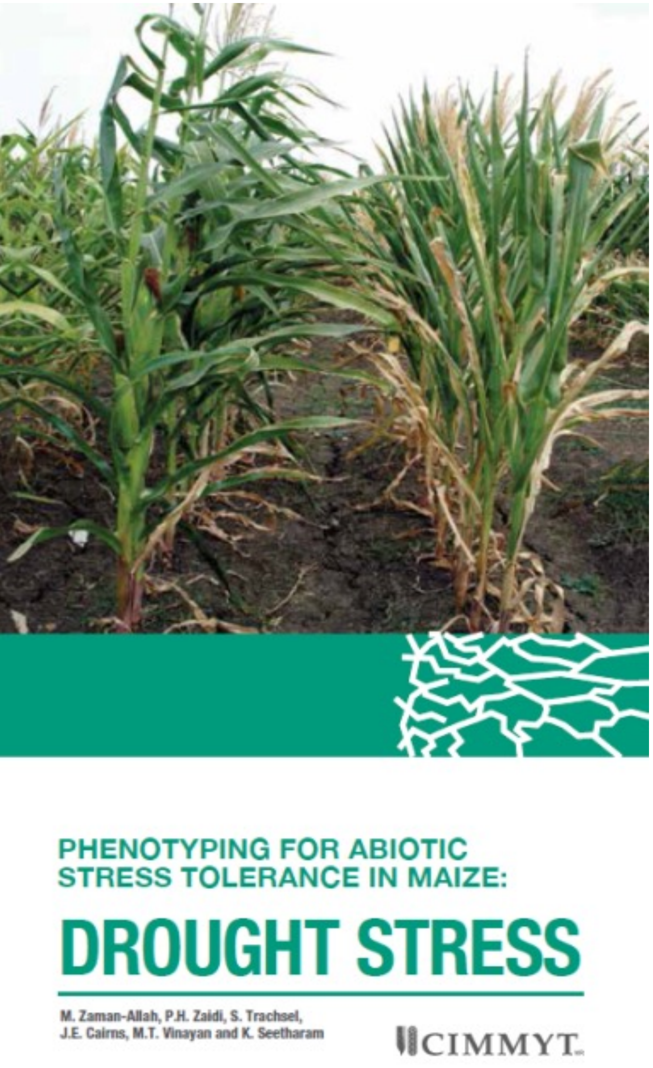


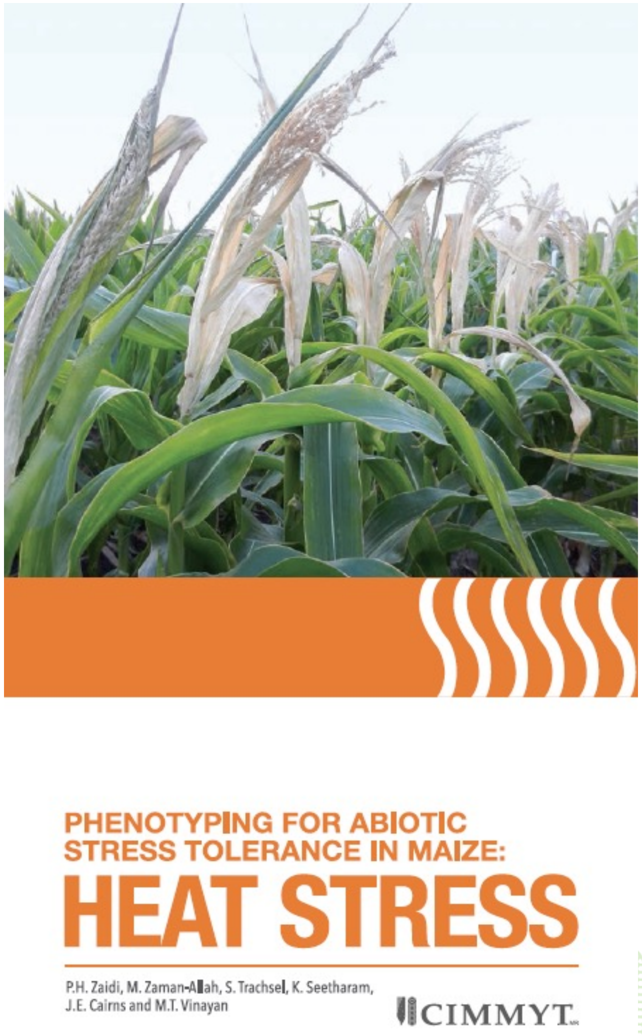
Masuka et al. 2012

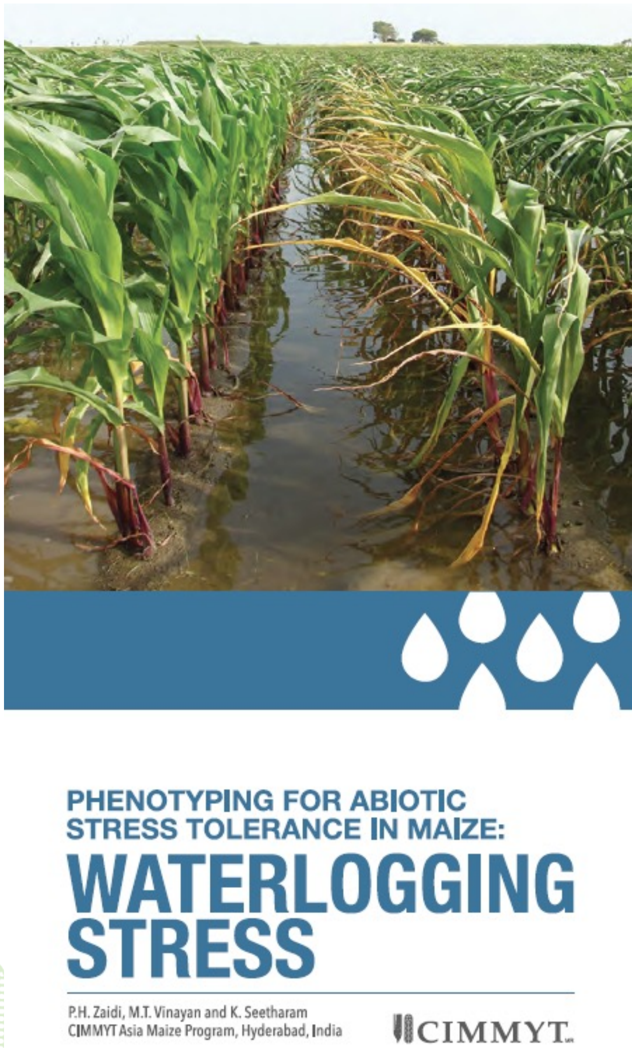


Das and Crossa

Harmonized phenotyping protocols for abiotic stresses







Conclusions

- **Large multilocation networks**
- **Emphasis in site quality – logistics for particular stresses**
- **Flexible phenotyping platforms**
- **Affordable, high-throughput phenotyping technologies**

Acknowledgements

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