UAV (and other) Phenotyping in an Arid Environment

Rick Ward, University of Arizona

The 4th Annual - Nordic Plant Phenotyping Network Workshop

Rungstedgaard - Rungsted, Denmark - November 22nd – 23rd, 2018

http://phenome2019.org/

contributors

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

TERRA-REF: ADVANCED FIELD CROP ANALYTICS

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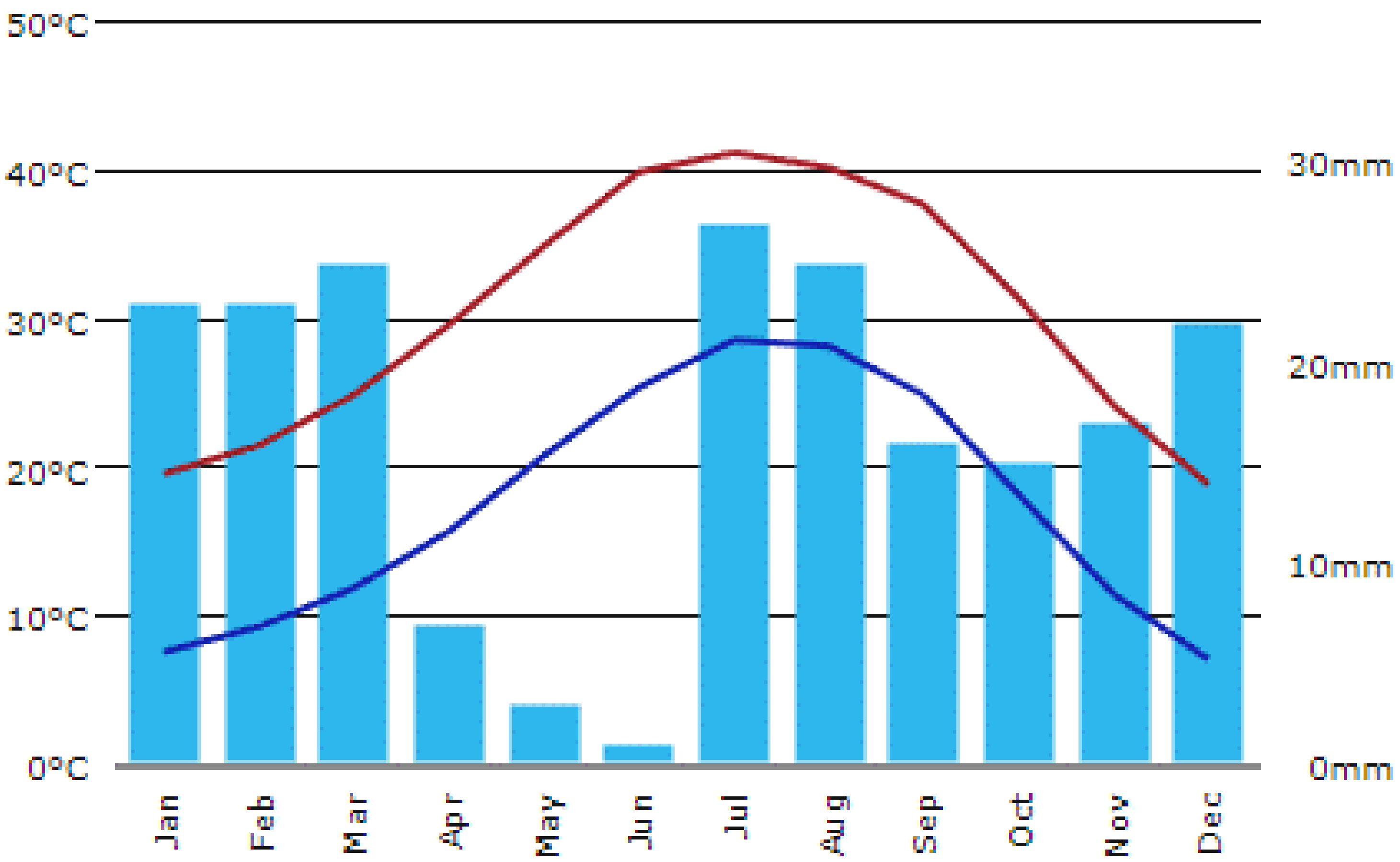
The Bud Antle Endowed Chair

Phoenix Drone Service

- M Newcomb
- J White
- A French
- K Khanal
- R. Wing
- D. Pauli
- P. Andrade
- J. Heun
- R. Tuberoso
- Giuseppe Emanuele Condorelli
- Geoff Morris and Zhenbin Hu
- Vasit Sagan’s lab
- M Tuller’s lab

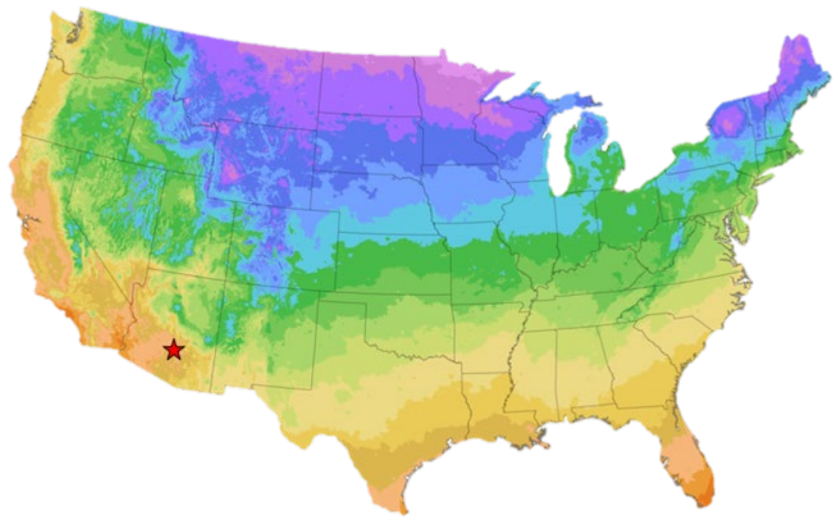
Maricopa, Arizona

Phoenix Climate Graph - Arizona Climate Chart



Phoenix weather averages

Annual high temperature:	30.3°C
Annual low temperature:	17.4°C
Average temperature:	23.85°C
Average annual precipitation - rainfall:	204 mm
Days per year with precipitation - rainfall:	36 days
Annual hours of sunshine:	3832 hours
Av. annual snowfall:	-



Intro

Photo credits: M Newcomb

Thoughts……..

- UAV data are Remote Sensing data
  - The discipline of Remote Sensing is rich with parallels
- QGIS and ArcGIS enable:
  - Polygons (one per plot) to be easily generated at start of season, stored in a single set of "shape" files (*.shp files)
  - A shape file includes a spreadsheet-like 'attribute table' with one row per polygon = one row per plot
  - GIS software enables near-instant generation of *plot level means*, stdevs, etc.
    - New *data* become new columns=traits in the attribute table
  - Field Book-like plot attributes- field address, genotype, experiment, row, column, rep, etc. are all in the attribute table
  - Copy/paste, or export/import into your statistical software and get your predictions.
  - These become data you can react to within hours and days of acquisition.
  - Time series translate into rates readily.

Thoughts……..2

- Establish georeferenced Ground Control Points (GCPs), with X,Y, and Z values at season's start.
- Use your GCPs to ensure your orthomosaics are "georectified", i.e. perfectly placed on the earth
  - Scott Chapman can provide work flows
- Go with both RGB (for visual guide for georectification and overall inspection), <u>and</u> Multi-spec
  - Work to show RGB is sufficient is relevant to LDC country situations

Frontiers paper

ORIGINAL RESEARCH ARTICLE

Front. Plant Sci., 26 June 2018 | https://doi.org/10.3389/fpls.2018.00893

Check for updates

Comparative Aerial and Ground Based High Throughput Phenotyping for the Genetic Dissection of NDVI as a Proxy for Drought Adaptive Traits in Durum Wheat

Giuseppe E. Condorelli[1], Marco Maccaferri[1*], Maria Newcomb[2], Pedro Andrade-Sanchez[2], Jeffrey W. White[3], Andrew N. French[3], Giuseppe Sciara[1], Rick Ward[2] and Roberto Tuberosa[1]

[1]Department of Agricultural Sciences, University of Bologna, Bologna, Italy

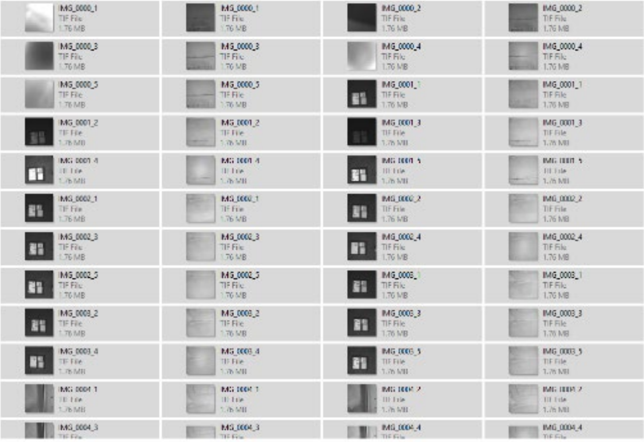
[2]Maricopa Agricultural Center, University of Arizona, Tucson, AZ, United States

[3]US Arid Land Agricultural Research Center, USDA-ARS, Maricopa, AZ, United States

High-throughput phenotyping platforms (HTPPs) provide novel opportunities to more effectively dissect the genetic basis of drought-adaptive traits. This genome-wide association study (GWAS) compares the results obtained with two Unmanned Aerial Vehicles (UAVs) and a ground-based platform used to measure Normalized Difference Vegetation Index (NDVI) in a panel of 248 elite durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) accessions at different growth stages and water regimes. Our results suggest increased ability of aerial over ground-based platforms to detect quantitative trait loci (QTL) for NDVI, particularly under terminal drought stress, with 22 and 16 single QTLs detected, respectively, and accounting for 89.6 vs. 64.7% phenotypic variance based on multiple QTL models. Additionally, the durum panel was investigated for leaf chlorophyll content (SPAD), leaf rolling and dry biomass under terminal drought stress. In total, 46 significant QTLs affected NDVI across platforms, 22 of which showed concomitant effects on leaf greenness, 2 on leaf rolling and 10 on biomass. Among 9 QTL hotspots on chromosomes 1A, 1B, 2B, 4B, 5B, 6B, and 7B that influenced NDVI and other drought-adaptive traits, 8 showed *per se* effects unrelated to phenology.

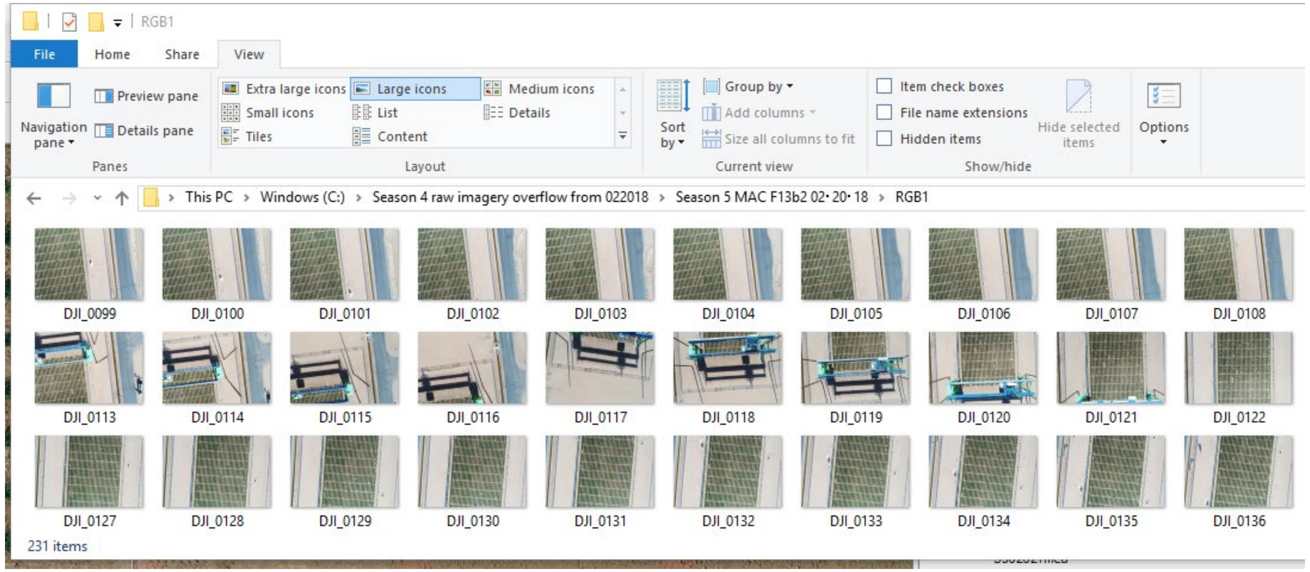
Copter work flow

Photo credits: M Newcomb



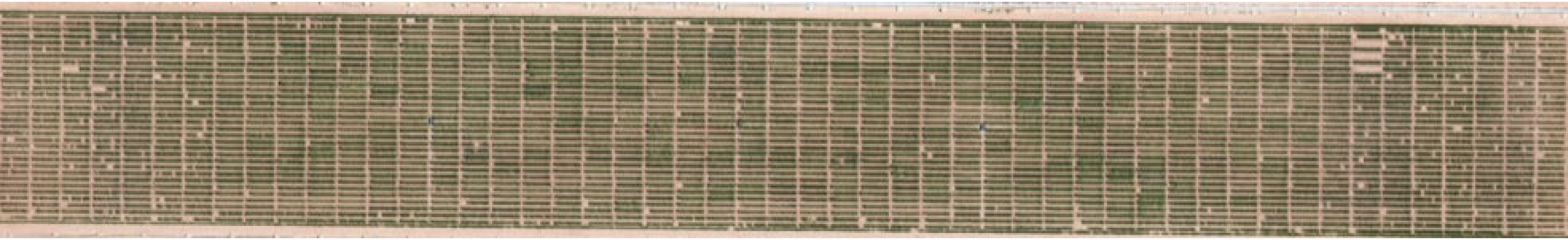
For 0.4 ha with 5 band camera, ~ 1800 images (3GB)

Post flight processing



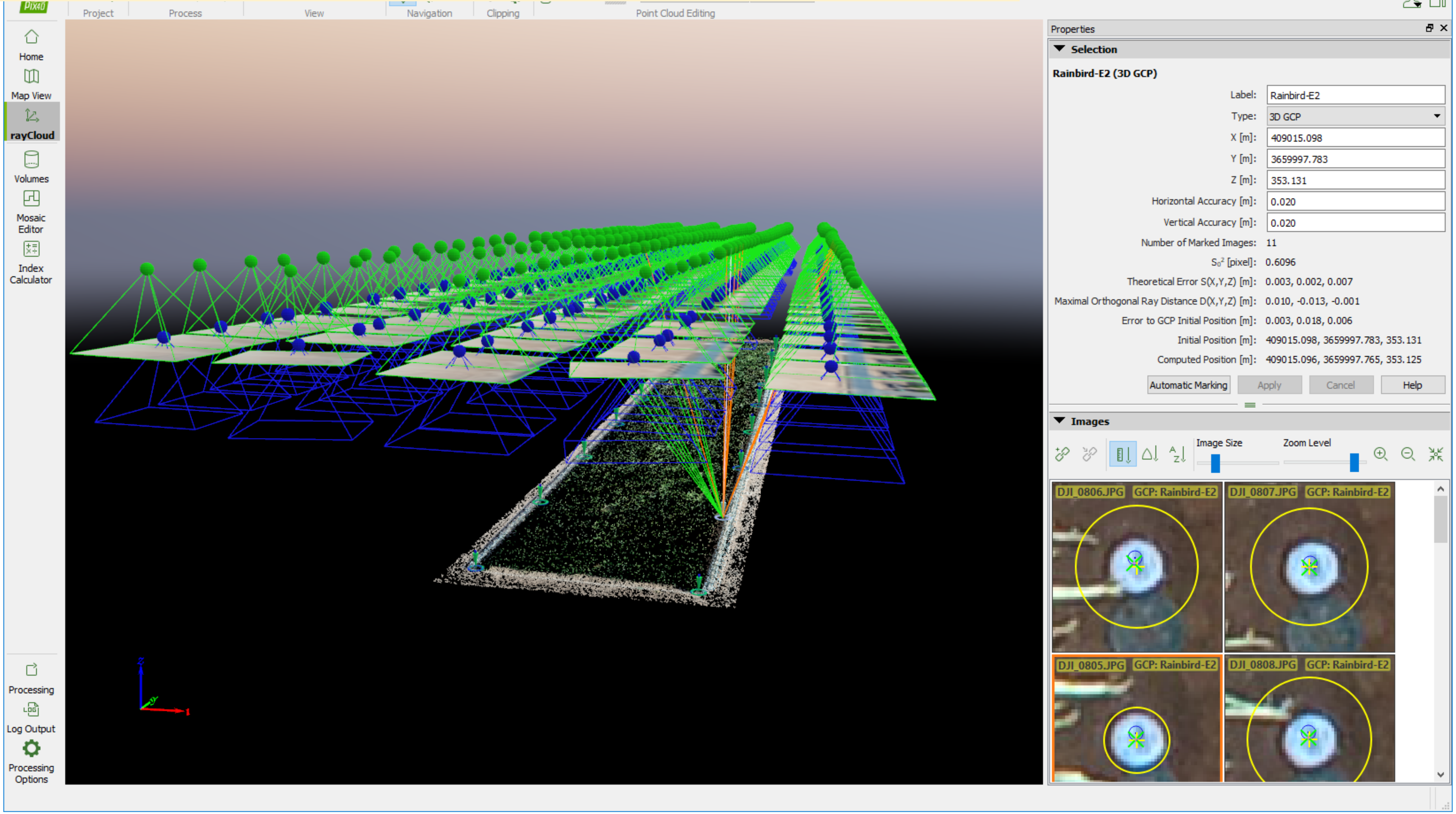
Original imagery

Pix4D, Agisoft, etc.

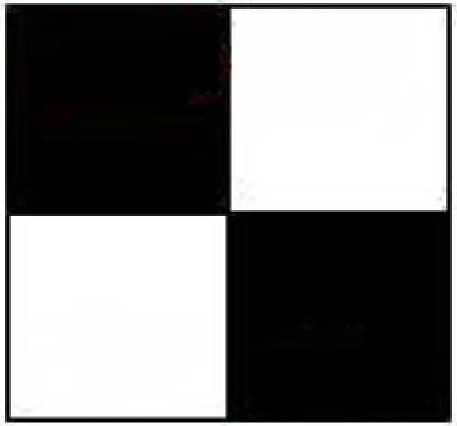


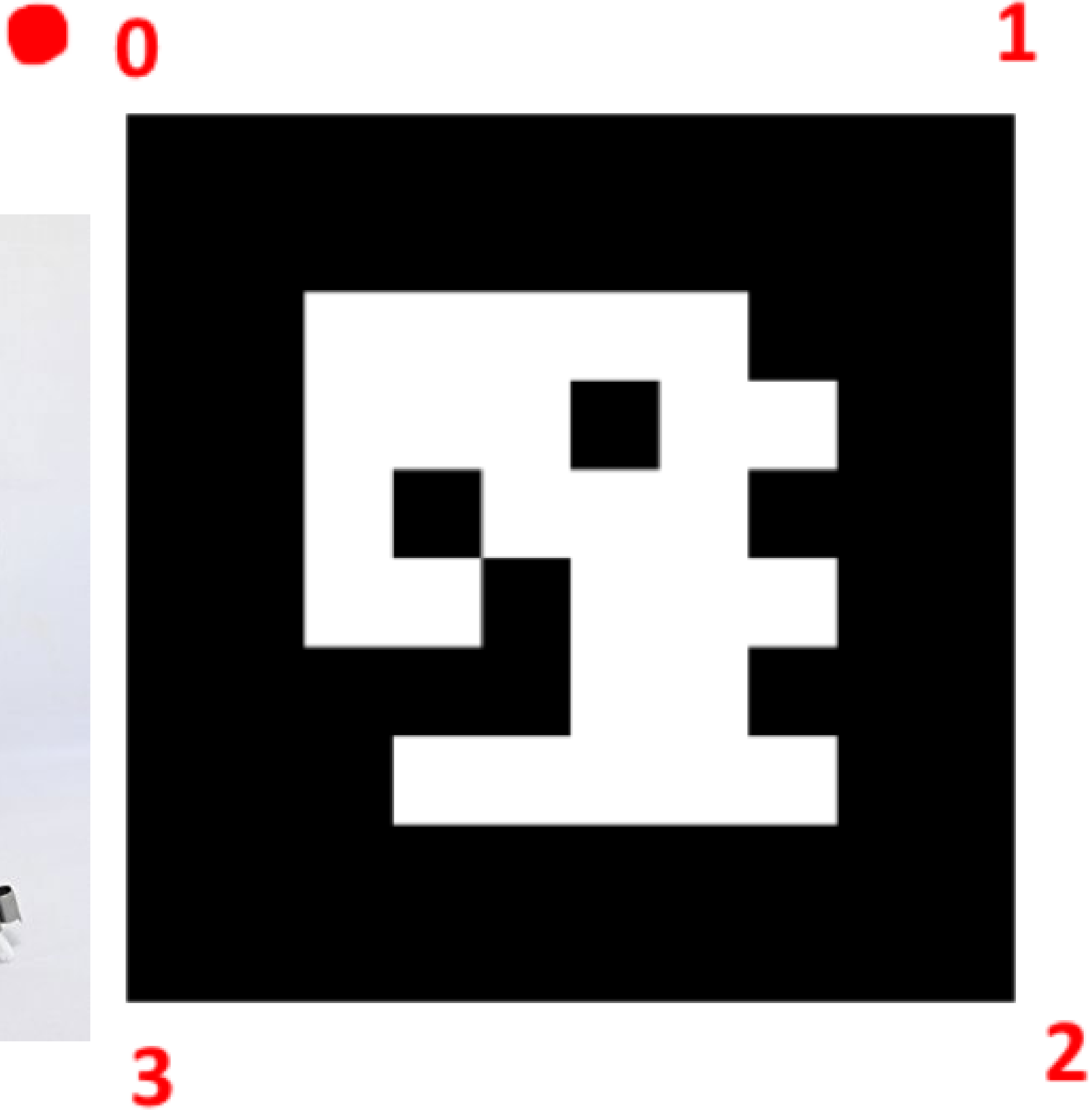
Geo-rectified Orthomosaic: Whether from RBG, multi-spectral, or thermal, each of the orthomoasic's pixels each contain 1 or more channels (layers) of data

rayCloud with one GCP selected and cameras visible



GCPs: Visible in images;
RTK precision located

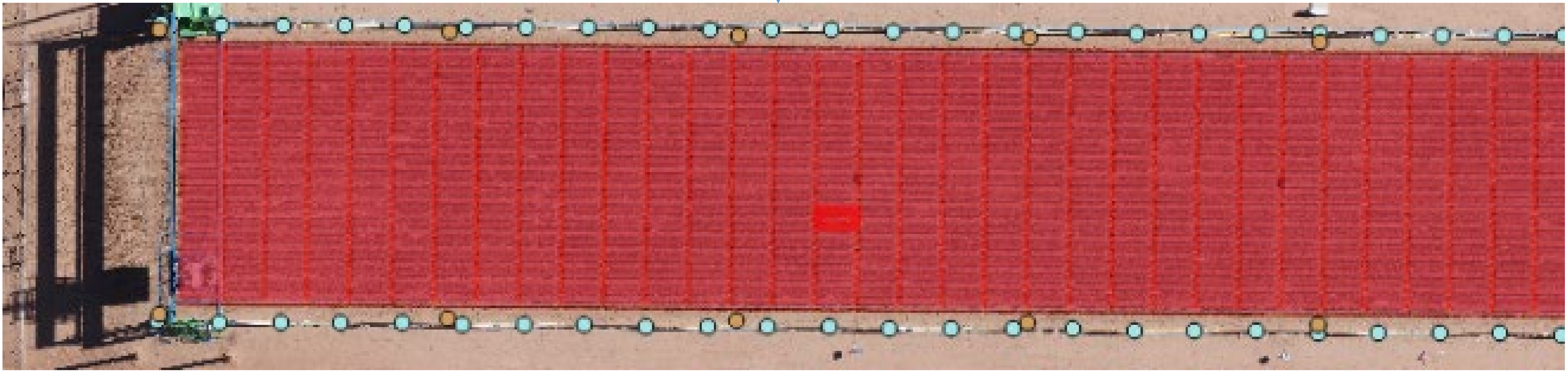




GCPs

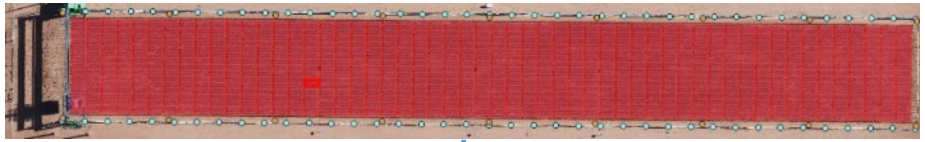
Post flight processing

Into GIS software
(click and drag)

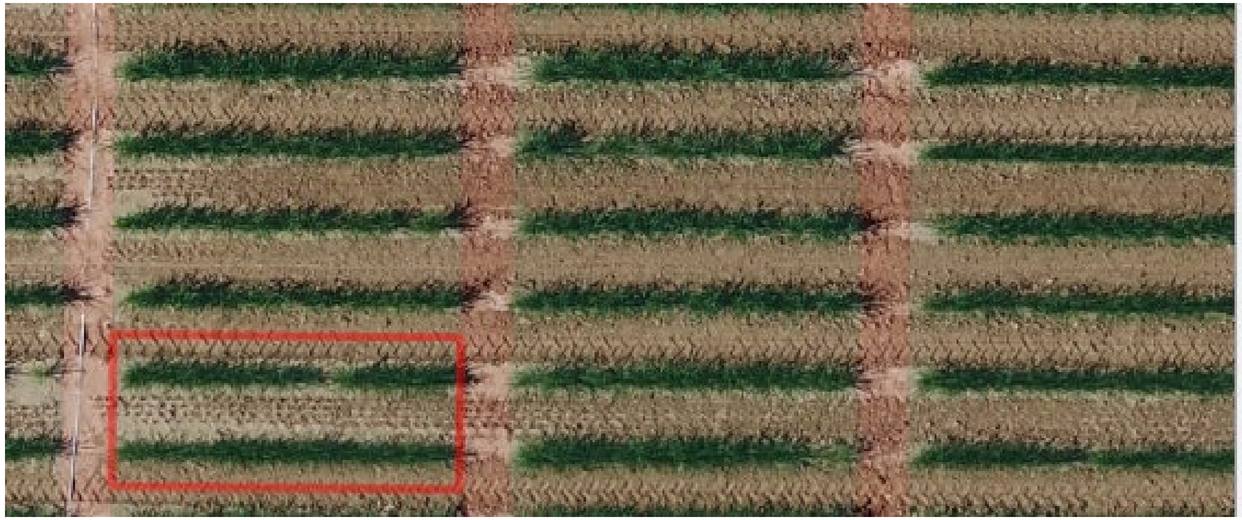


Ortho as raster layer in QGIS with overlay of one polygon per plot (in a shape file)

Post flight processing



GIS Zonal statistics (stats by polygon=plot)



Above with aggregate information across layers for a single plot

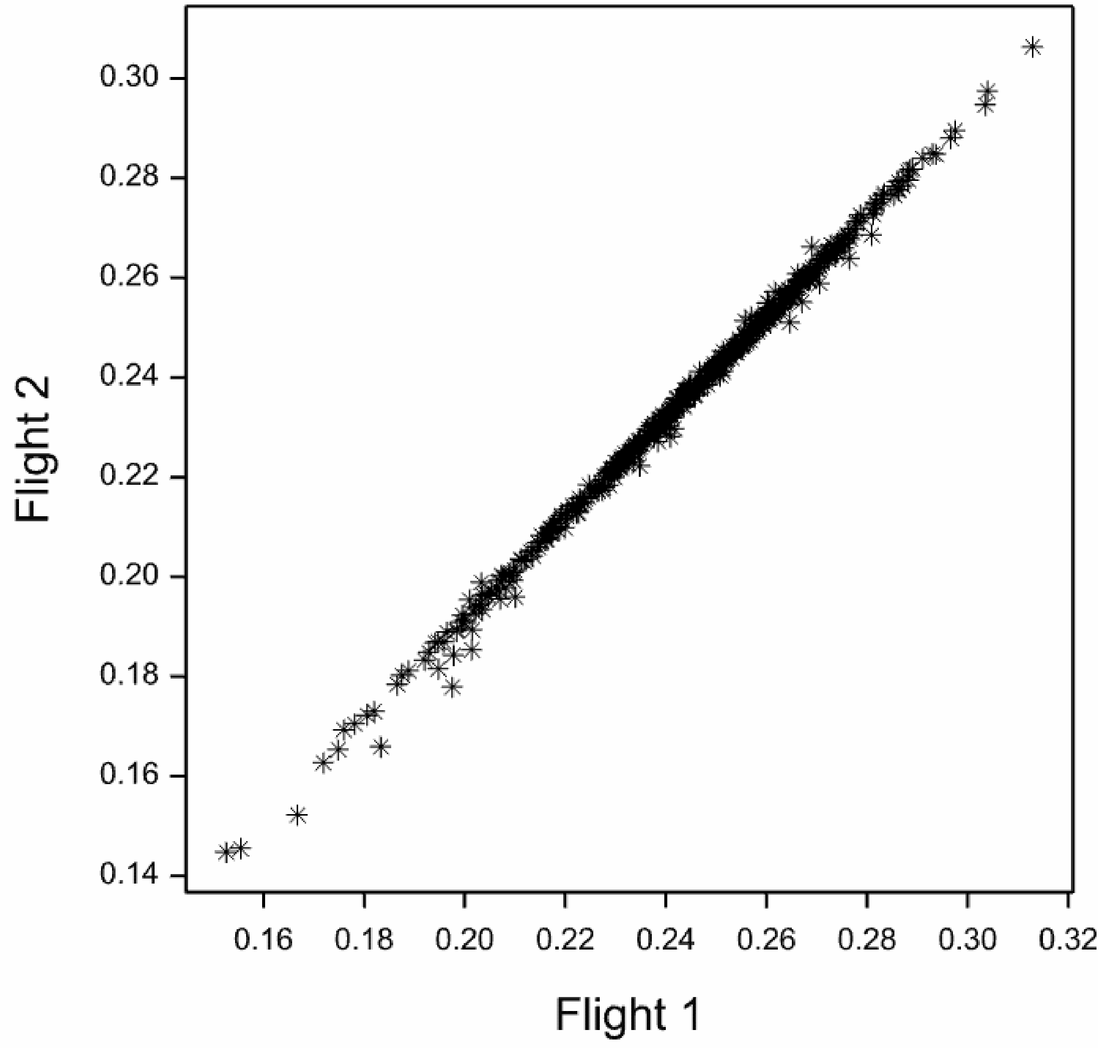
Feature	Value
wheatF13B2_2017_2018_2rowplots_rotaten5225_3660095_shift0017e	
Short_name	Astrodur
(Derived)	
(Actions)	
Id	614
range	39
column	6
replicate	2=North
Plot_type	Exp
site	MAC Field Scanner Season 5 Range 39 Column 6
cultivar	UNIBO Durum Panel DP_207
Durum_pane	DP_207
Short_name	Astrodur
Origin	AUSTRALIA
Entry_name	Astrodur
Design_Row	30
Design_Col	5

Attribute table (living field book- imagine it pivoted 90 degrees); one row per plot

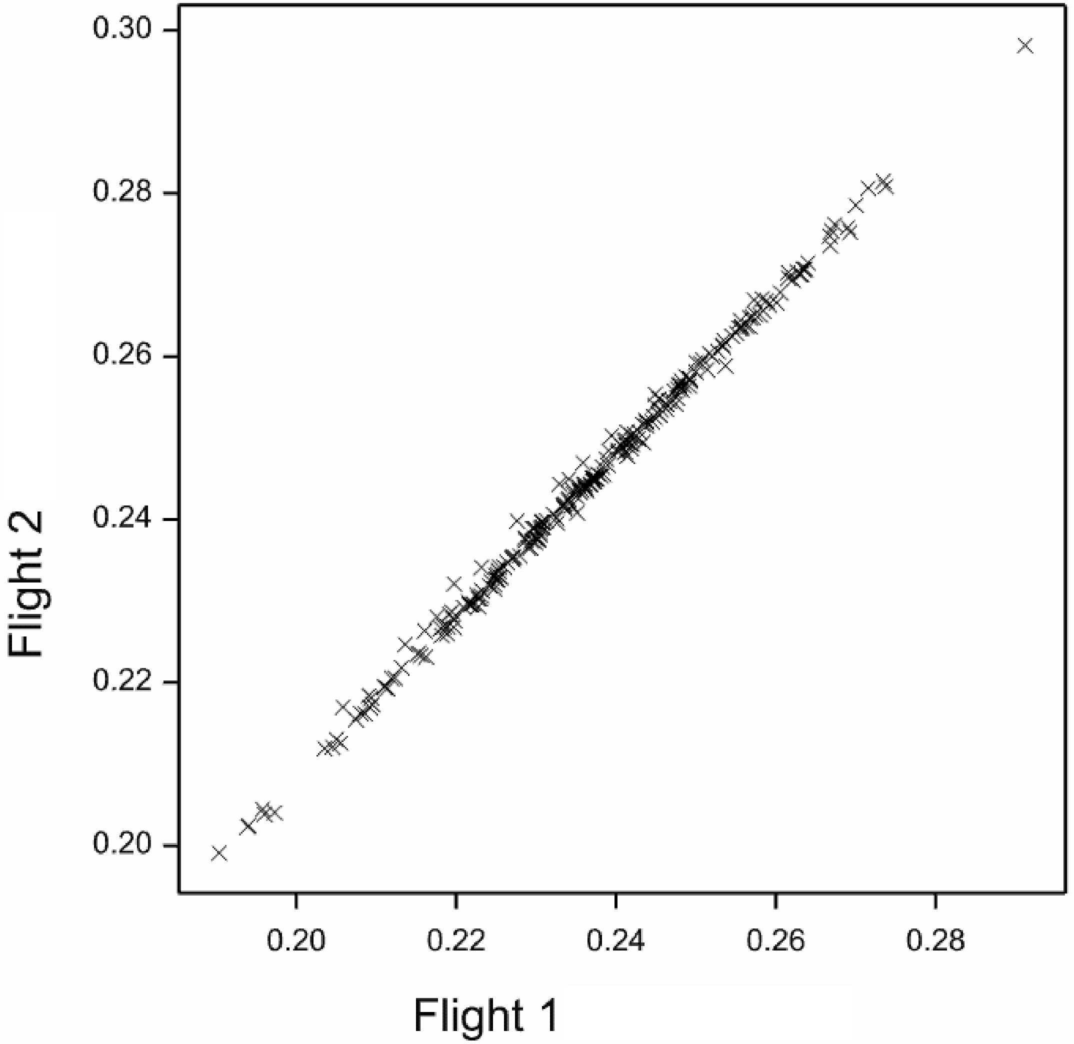
Repeatability

- 504 plots measured in 15 minutes x 2 flights

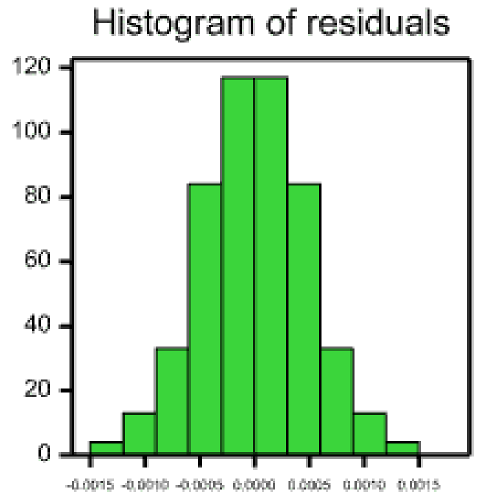
Plot means (n=504)

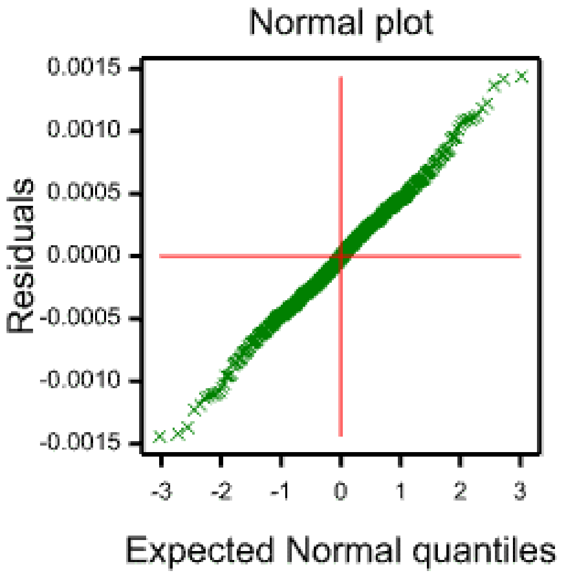


Genotype means (n=252)



Entry variation





Detects entry variation
Data properties are as expected for Normal residual distributions

Generalized heritability

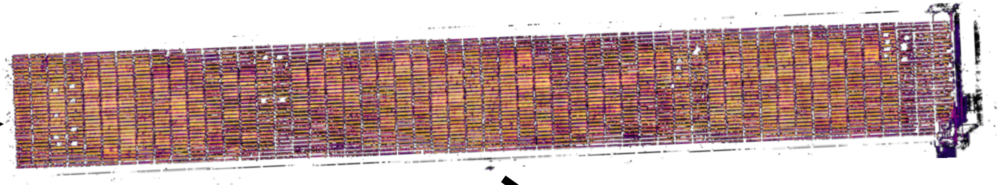
Term	Heritability
Durum_pane	0.6553

Genetic Experimental work flow

1

252 wheat accessions x 2 reps

2

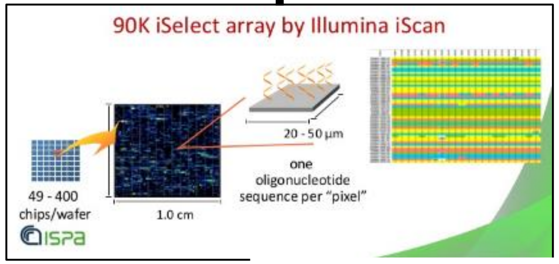


3

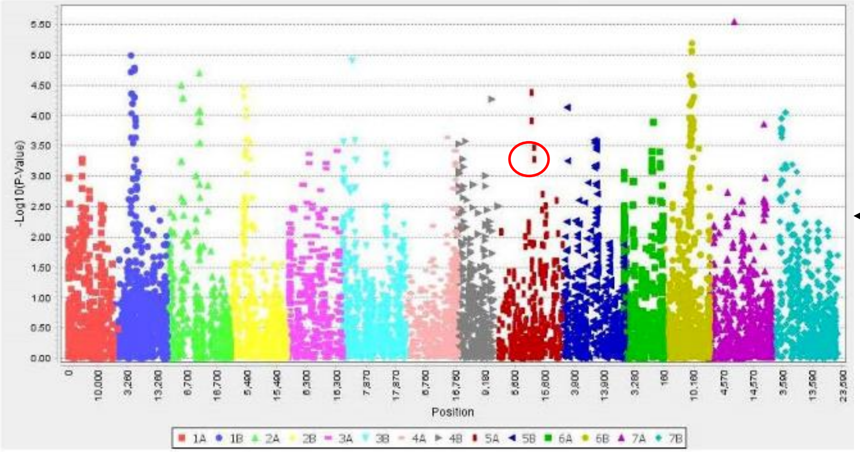
504 plot level spectral signatures (NDVI: only two bands: red and NIR); analyses in Genstat shows high generalized heritability.

4

SNP genotype data (Italy)



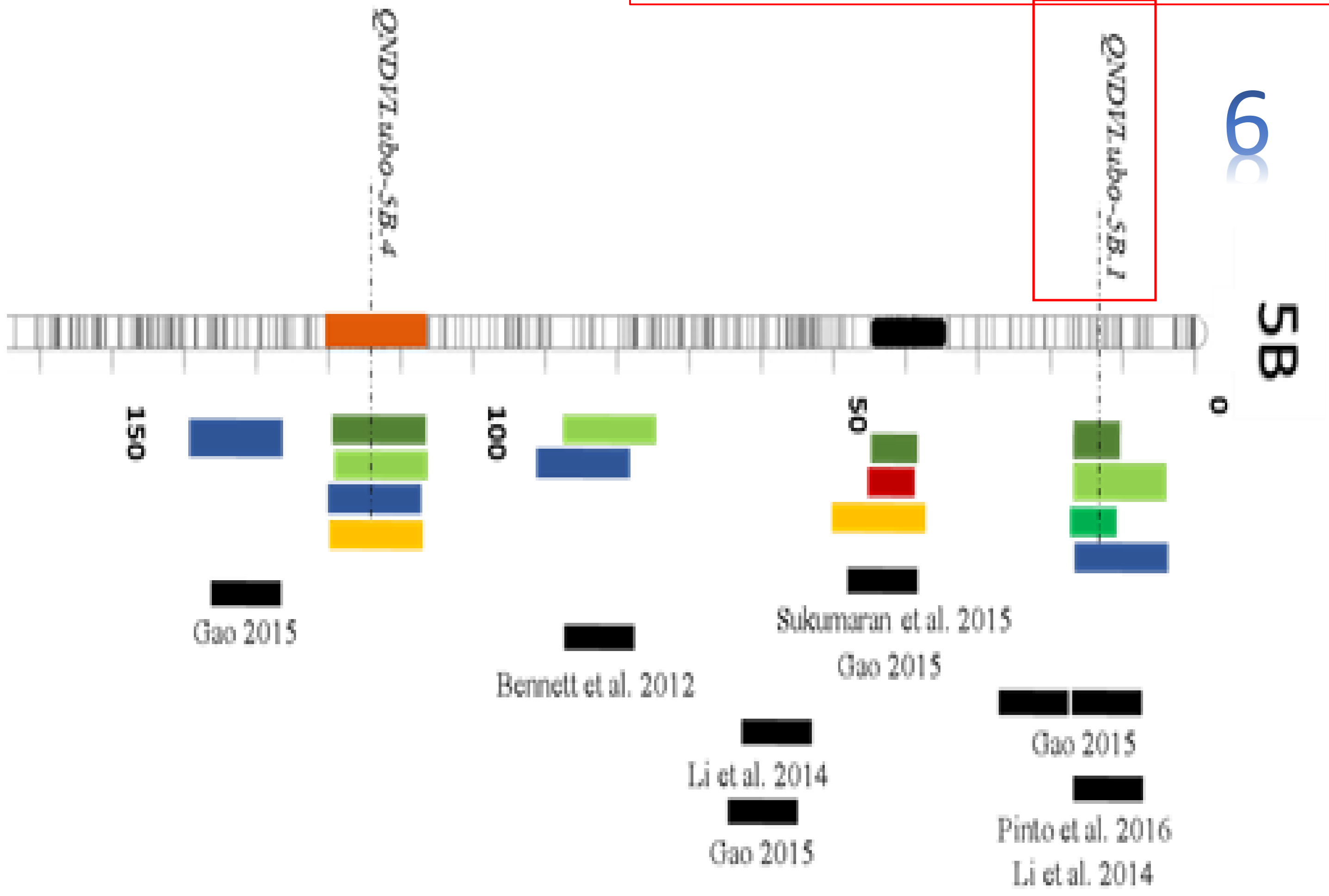
5



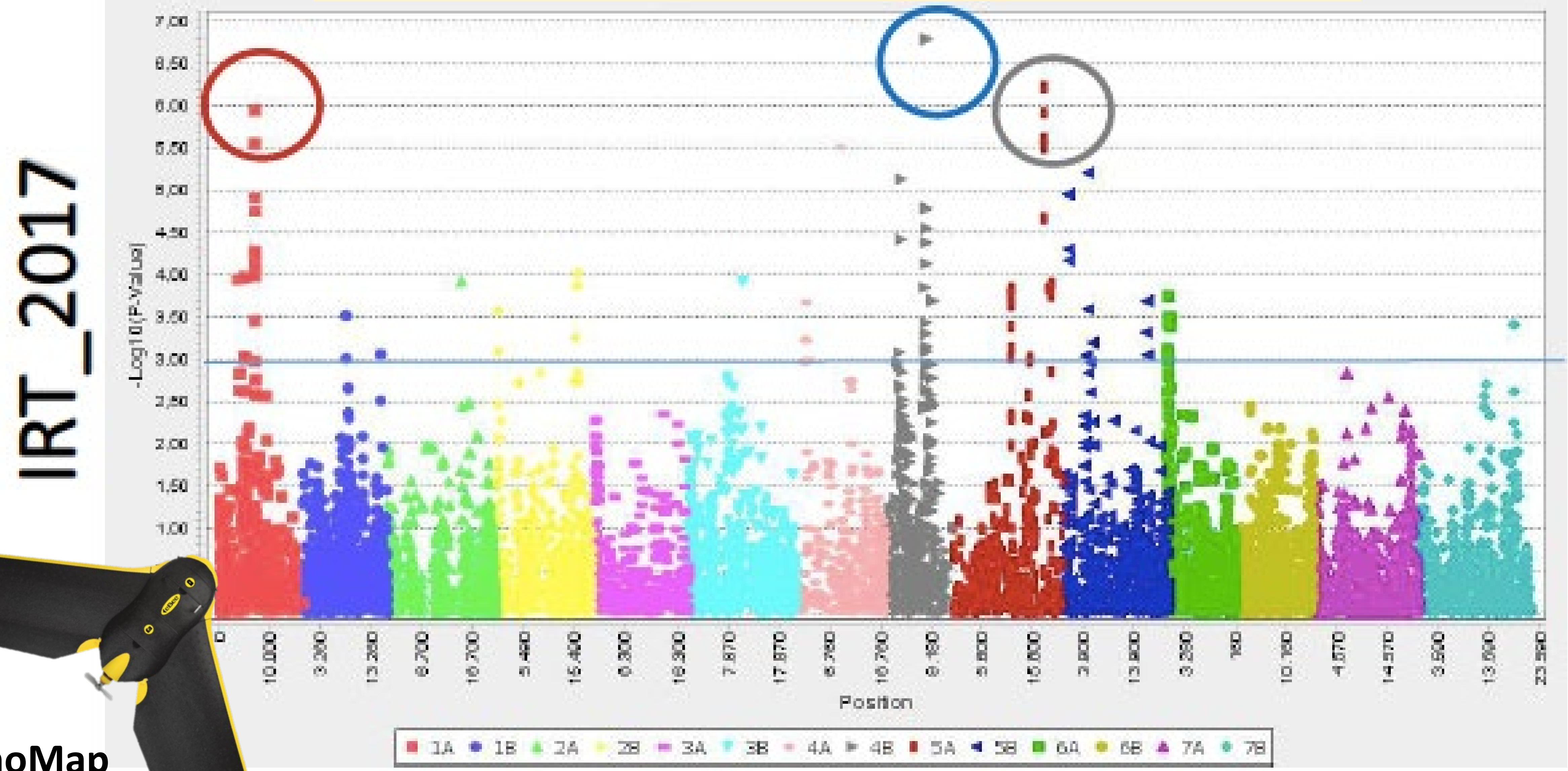
GWAS analysis of combined phenotypes and genotypes reveal chromosomal segments associated with NDVI ("high rises" >3.0 on Y axis)

Co-located qtl: $\boldsymbol{\sigma}^2_g$

QTL co-located with three previously located by other methods



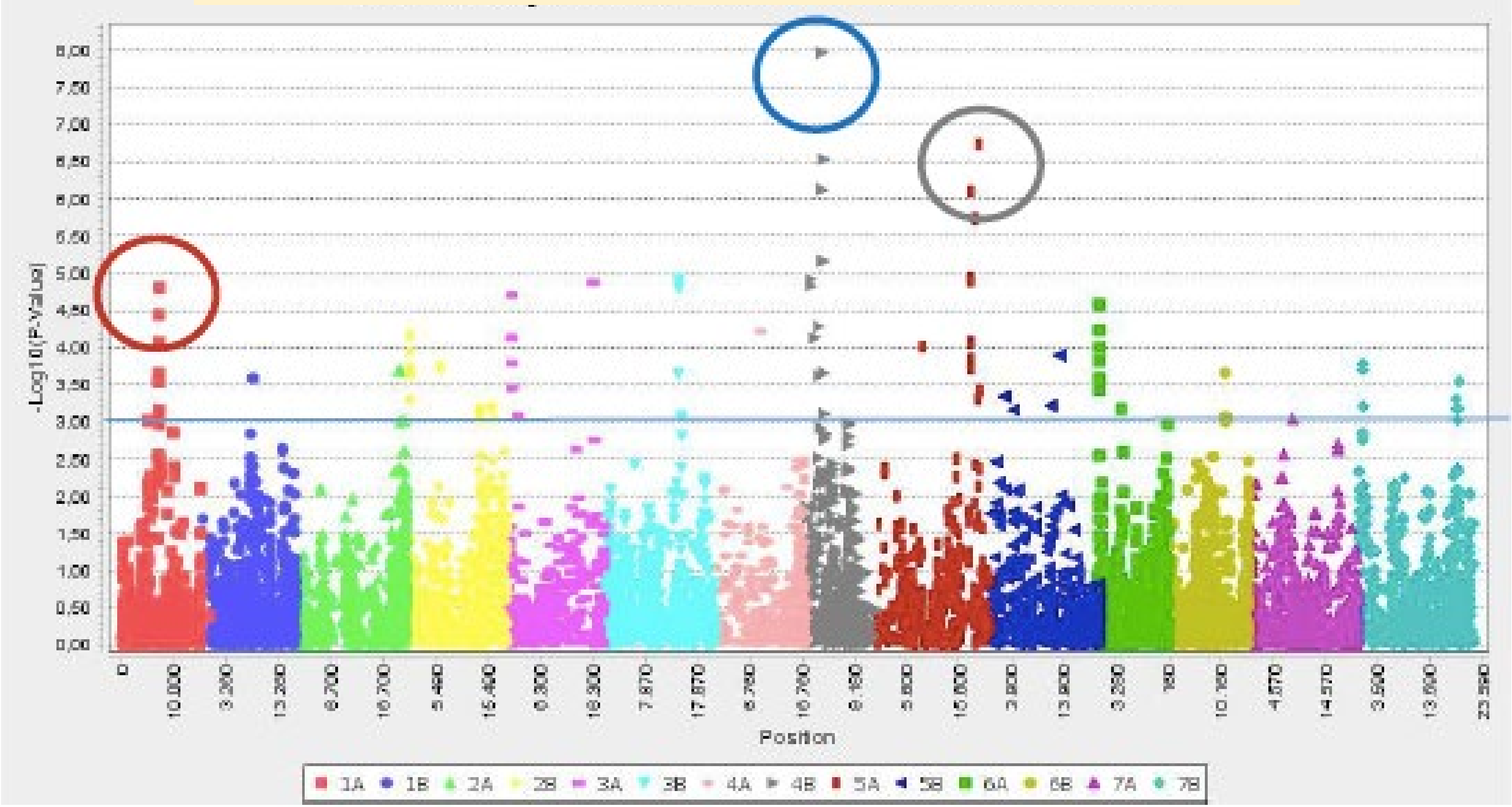
2 seasons of wheat IRT data point to same 3 QTL?



thermoMap

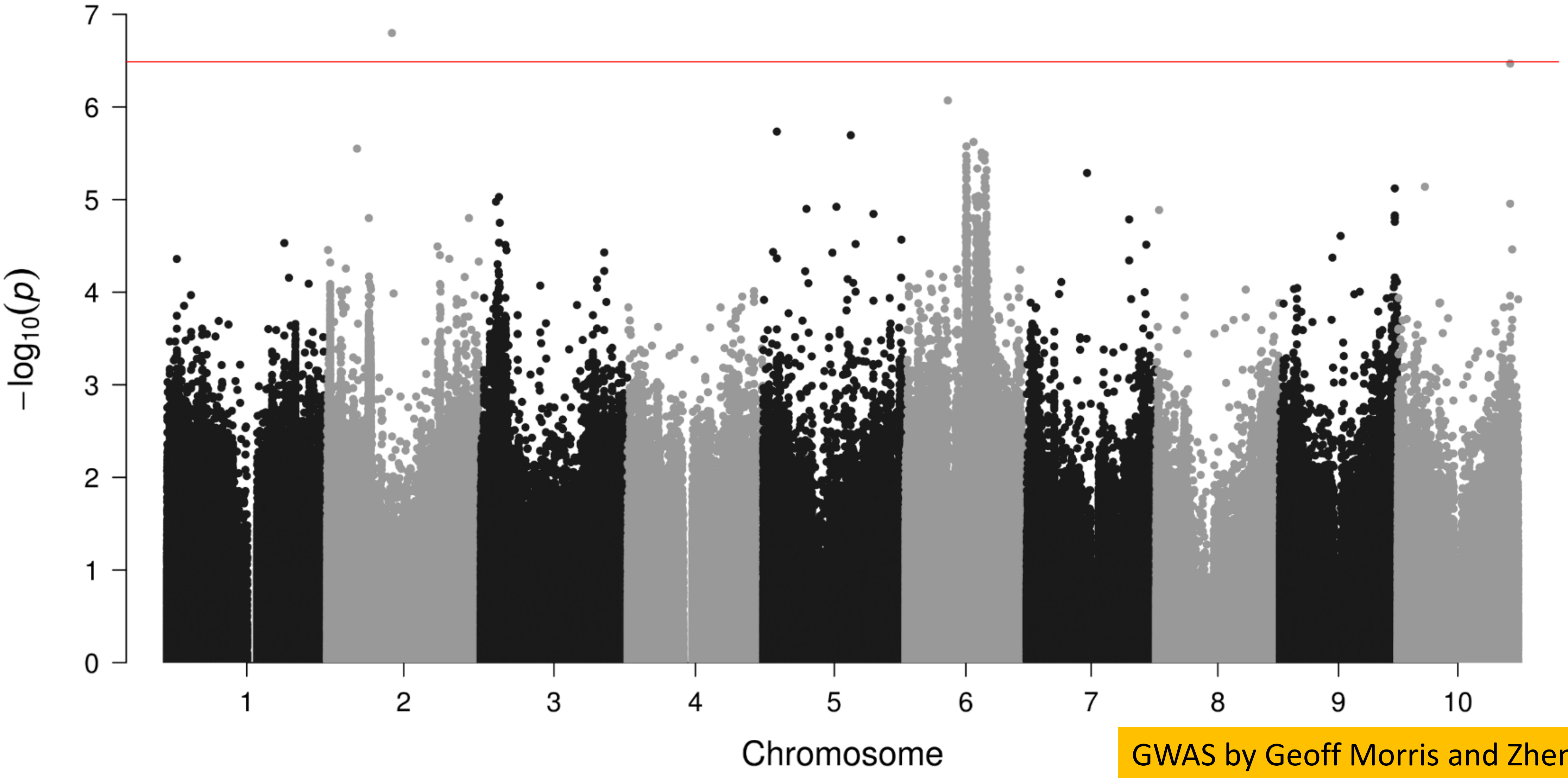
2 seasons of wheat IRT data point to same 3 QTL?

IRT_2018



Recent tests at Maricopa in cooperation with St Louis University with ICI, FLIR and Thermomap gave promising results showing moderately high general h^2 (>.6)

Early evidence of Canopy temperature qtl for Sorghum Bioenergy Associate Panel (BAP) using Ebee/Thermomap

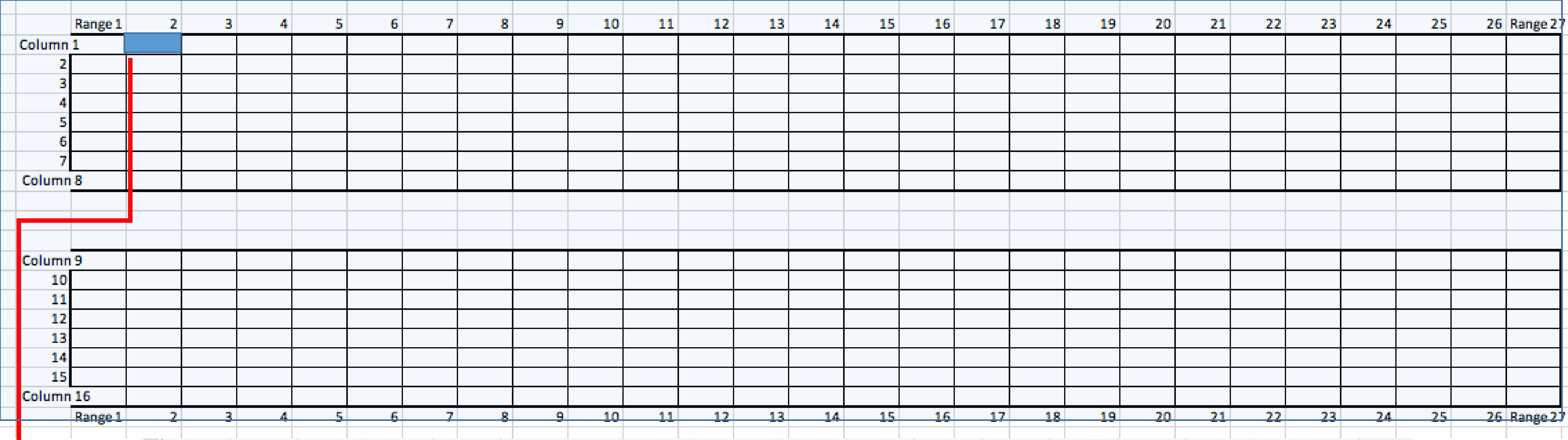


GWAS by Geoff Morris and Zhenbin Hu
Based on 9 million SNPS

Segregation for greenness in a well characterized population of 210 rice Recombinant Inbred Lines


Segregation
for greenness
in a well
characterized
rice RIL

Orthomosaic of GLI in a population of 220 rice RILS in 2 replications



1	RanCol	exp	year	trt	Duke_plot	rep	Des_row	Des_col	entry	type	F_range	F_column	ord_NS	ord_S	dir	GLIiMaskme
2	01_01	rice_ril	2018	WW	1001	1	1	1	R177	RIL	1	1	1	1	LR	0.121858373
3	01_02	rice_ril	2018	WW	1028	1	2	1	R169	RIL	1	2	2	2	LR	0.13691947
4	01_03	rice_ril	2018	WW	1055	1	3	1	R218	RIL	1	3	3	3	LR	0.092459875
5	01_04	rice_ril	2018	WW	1082	1	4	1	R023	RIL	1	4	4	4	LR	0.117663727
6	01_05	rice_ril	2018	WW	1109	1	5	1	R045	RIL	1	5	5	5	LR	0.153239622
7	01_06	rice_ril	2018	WW	1136	1	6	1	R184	RIL	1	6	6	6	LR	0.089187546
8	01_07	rice_ril	2018	WW	1163	1	7	1	R187	RIL	1	7	7	7	LR	0.056186825
9	01_08	rice_ril	2018	WW	1190	1	8	1	R002	RIL	1	8	8	8	LR	0.076185035
10	01_09	rice_ril	2018	WW	2001	2	9	1	R062	RIL	1	9	217	224	LR	0.05951574
11	01_10	rice_ril	2018	WW	2028	2	10	1	R195	RIL	1	10	218	223	LR	0.105752728
12	01_11	rice_ril	2018	WW	2055	2	11	1	R068	RIL	1	11	219	222	LR	0.178539737
13	01_12	rice_ril	2018	WW	2082	2	12	1	R210	RIL	1	12	220	221	LR	0.133648067
14	01_13	rice_ril	2018	WW	2109	2	13	1	R235	RIL	1	13	221	220	LR	0.127810261
15	01_14	rice_ril	2018	WW	2136	2	14	1	R007	RIL	1	14	222	219	LR	0.107340674
16	01_15	rice_ril	2018	WW	2163	2	15	1	R197	RIL	1	15	223	218	LR	0.098848943
17	01_16	rice_ril	2018	WW	2190	2	16	1	R150	RIL	1	16	224	217	LR	0.062127226
18	02_01	rice_ril	2018	WW	1002	1	1	2	R006	RIL	2	1	9	16	RL	0.131555065
19	02_02	rice_ril	2018	WW	1029	1	2	2	R120	RIL	2	2	10	15	RL	0.115000995

Attrributes from Shape file | 2D field map | +

Ready

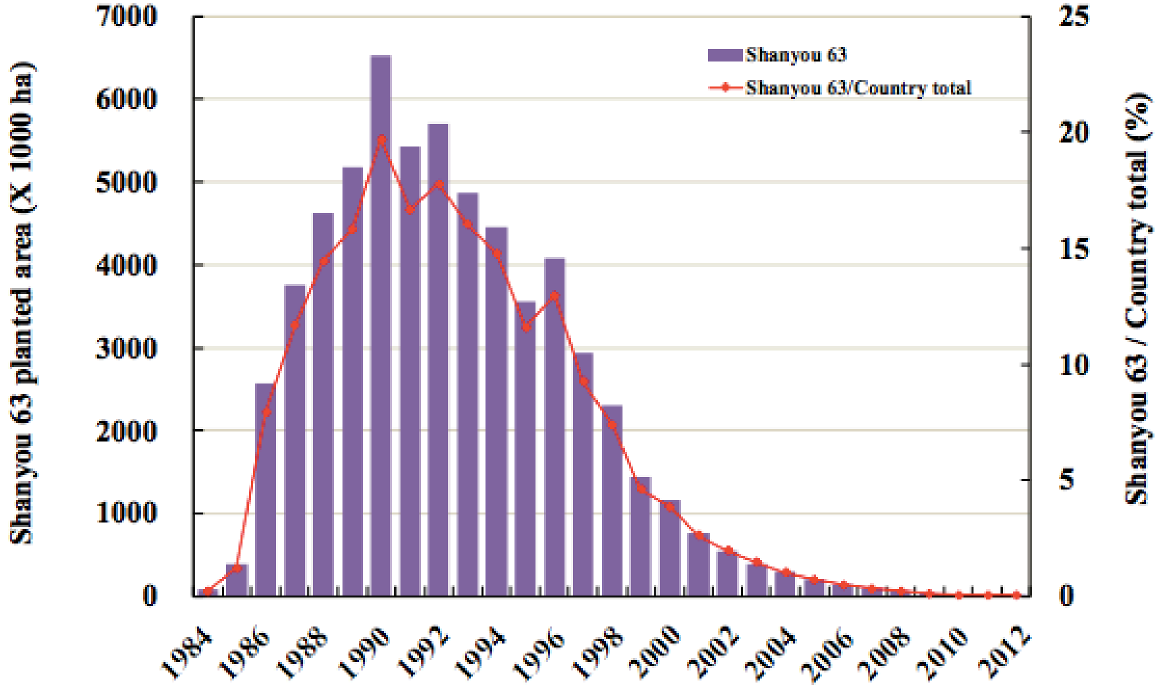
QGIS

Sequenced parents of rice hybrid parent genomes

A

Fig. S1. Some features of ZS97, MH63 and their hybrid SY63.

C



PNAS

Extensive sequence divergence between the reference genomes of two elite *indica* rice varieties Zhenshan 97 and Minghui 63

Jianwei Zhang (张建伟)[a,b,c,1], Ling-Ling Chen (陈玲玲)[a,1], Feng Xing (邢锋)[a,1], David A. Kudrna[b,c], Wen Yao (姚文)[a], Dario Copetti[b,c,d], Ting Mu (穆婷)[a], Weiming Li (李伟明)[a], Jia-Ming Song (宋佳明)[a], Weibo Xie (谢为博)[a], Seunghee Lee[b,c], Jayson Talag[b,c], Lin Shao (邵林)[a], Yue An (安玥)[a], Chun-Liu Zhang (张春柳)[a], Yidan Ouyang (欧阳亦聃)[a], Shuai Sun (孙帅)[a], Wen-Biao Jiao (焦文标)[a], Fang Lv (吕芳)[a], Bogu Du (杜博贾)[a], Meizhong Luo (罗美中)[a], Carlos Ernesto Maldonado[b,c], Jose Luis Goicoechea[b,c], Lizhong Xiong (熊立仲)[a], Changyin Wu (吴昌银)[a], Yongzhong Xing (邢永忠)[a], Dao-Xiu Zhou (周道绣)[a], Sibin Yu (余四斌)[a], Yu Zhao (赵毓)[a], Gongwei Wang (王功伟)[a], Yeisoo Yu[b,c,2], Yijie Luo (罗艺洁)[a], Zhi-Wei Zhou (周智伟)[a], Beatriz Elena Padilla Hurtado[b,c], Ann Danowitz[b], Rod A. Wing[b,c,d,3], and Qifa Zhang (张启发)[a,3]

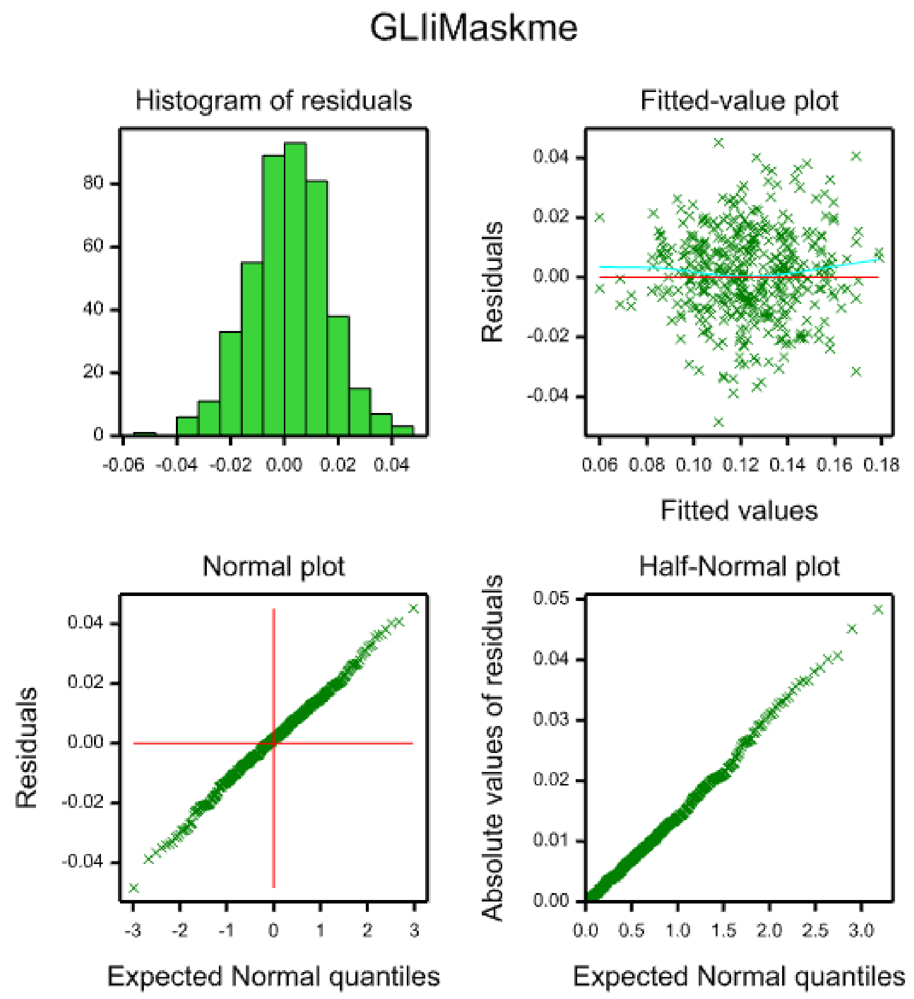
[a]National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China; [b]Arizona Genomics Institute, School of Plant Sciences, University of Arizona, Tucson, AZ 85721; [c]BIO5 Institute, School of Plant Sciences, University of Arizona, Tucson, AZ 85721; and [d]International Rice Research Institute, Genetic Resource Center, Los Baños, Laguna, Philippines

Entry variance is positive

Heritability: 0.7475

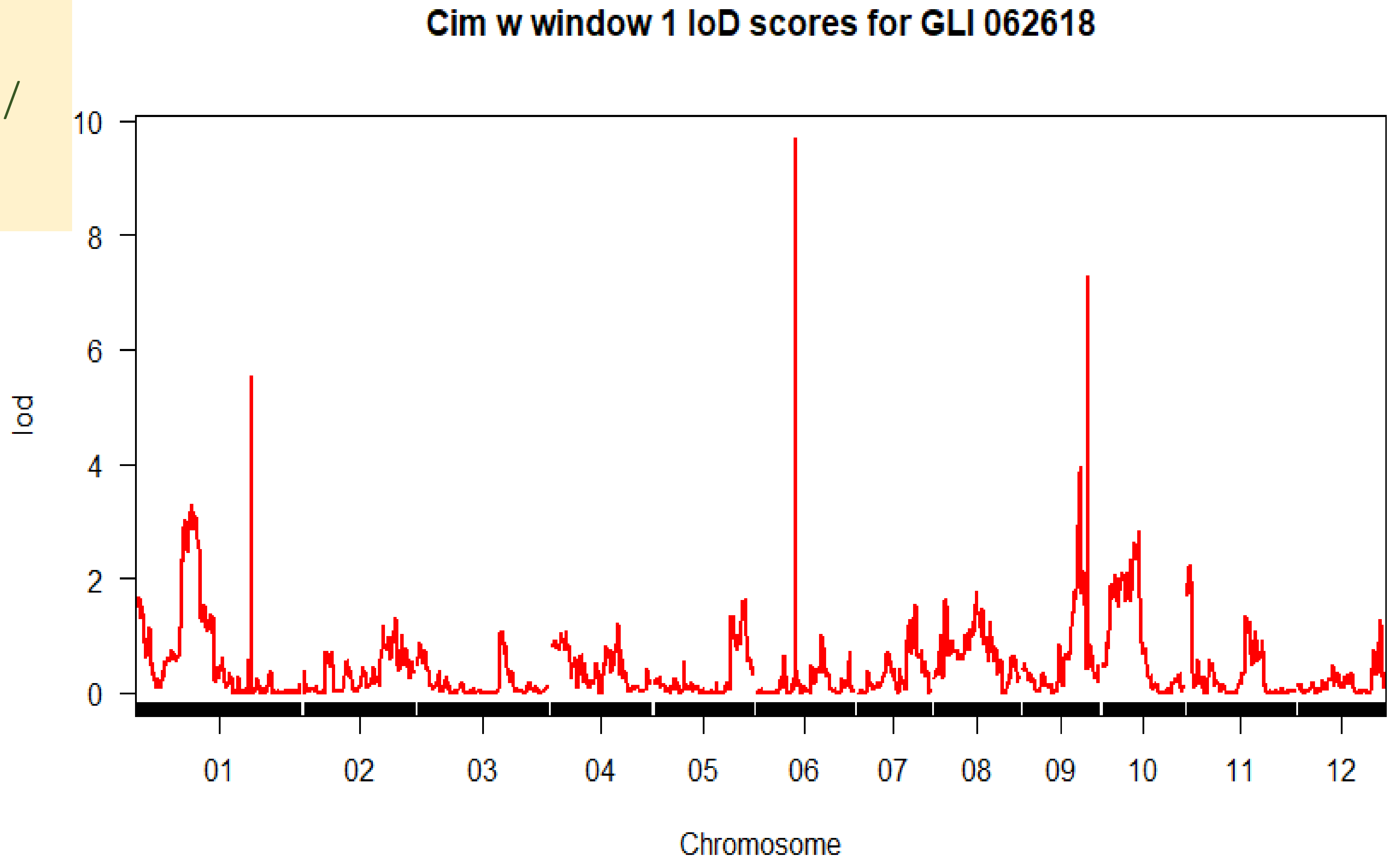
LSD @ 0.01 alpha =0.015;

Fixed term	**Wald statistic**	**F pr**
entry	2567.92	<0.001

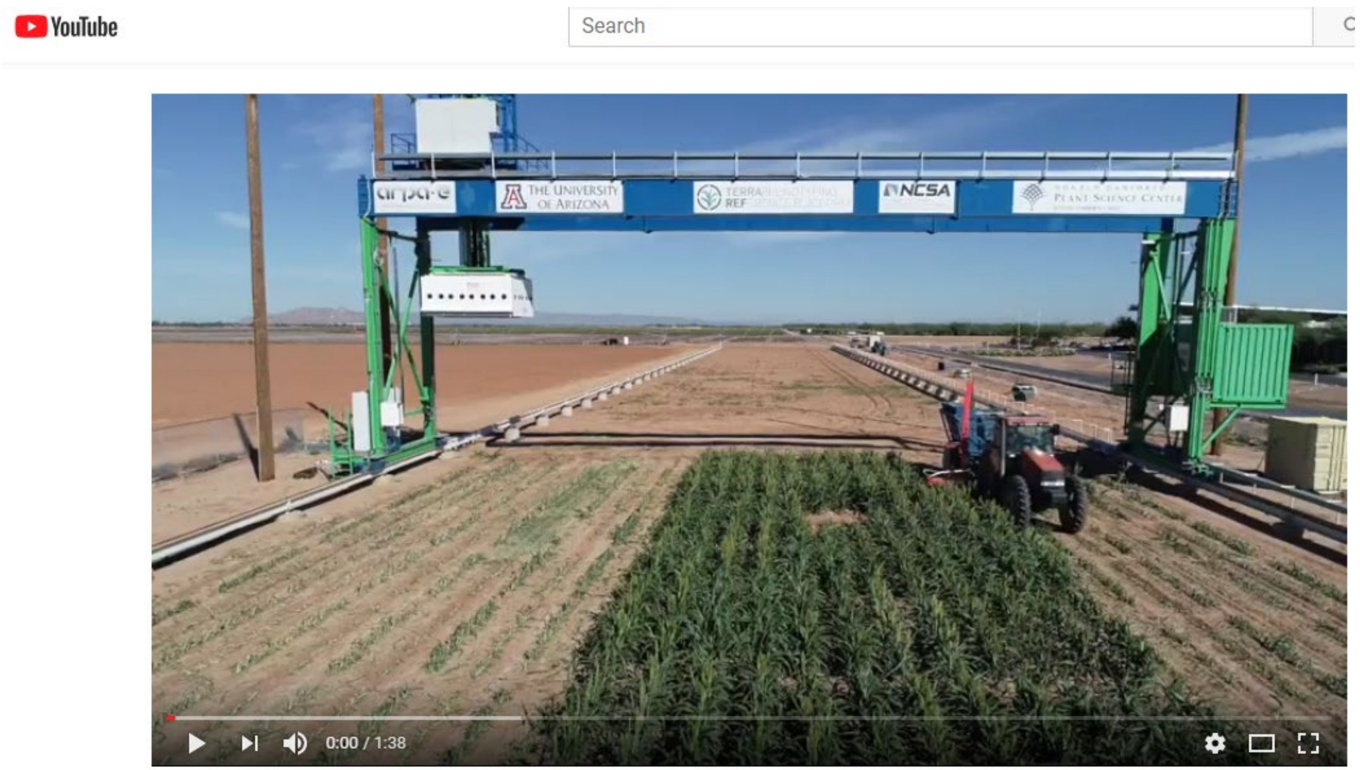


QTL in rice for "Green Leaf Index"

GLI = (2 x G - R - B) / (2 x G+R+B)



Field Scanalyzer (click picture to see the movie)



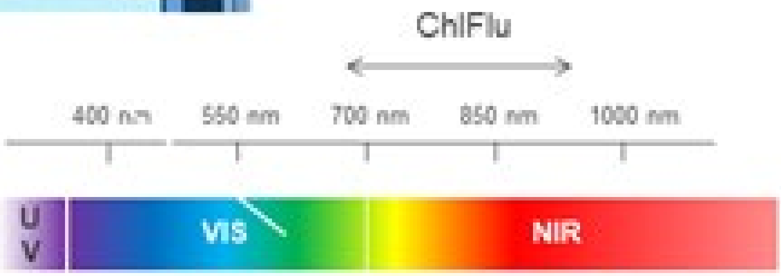
Video credits: K Khanal

Phenotyping sensors

- 2x 9MP RGB Camera
- FLIR thermal camera
- PSII camera (Kautsky effect)
- Laser scanners - special development by Fraunhofer IIS
    - 0.6 m Scan width
    - 1.5 m Scan depth (adjustable)
    - 0.25 mm point to point distance
    - 2x side view with different setup
- Hyperspectral cameras
- Active Reflectance Sensor (Crop Circle)
- NDVI Sensor
- Color Sensor

PSII fluorescence system

LemnaTec System:
Type: LED
Color: red
Intensity: 0-7000 µmol m-2 s-1 at 70 cm
Resolution: 1936*1216
Frame Rate: 100 images/s
Range: 690-730nm
Imaging area: 2000x1500mm



the fluorescence camera in the gantry camera box as it is set up now requires dark adaptation; the red flash duration is one second in total; F0 is measured 20 ms after the red flash first starts, and Fm is expected after 0.8-0.9 s. We've been running the instrument at 70% saturation, which is adjustable in the script

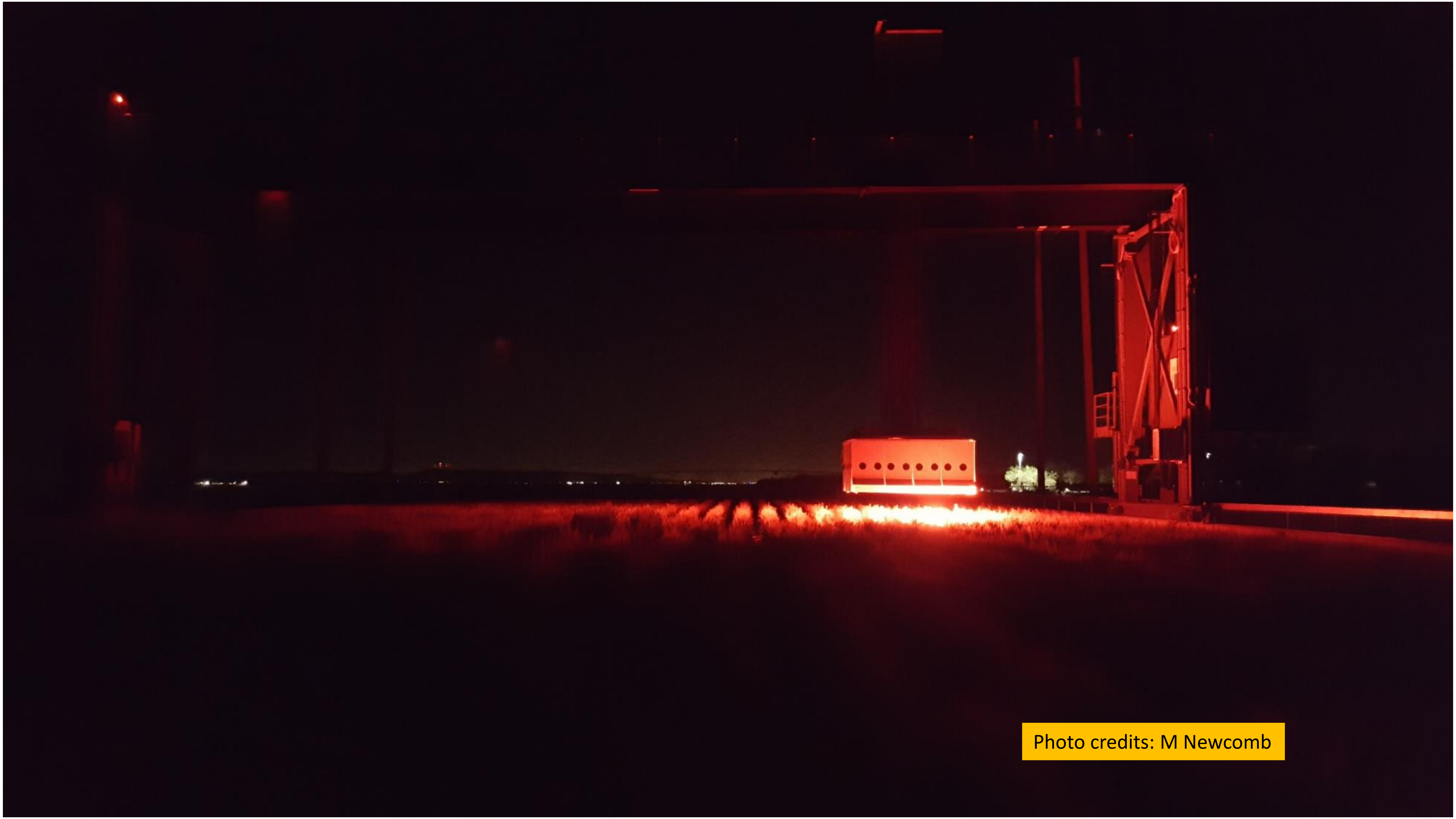
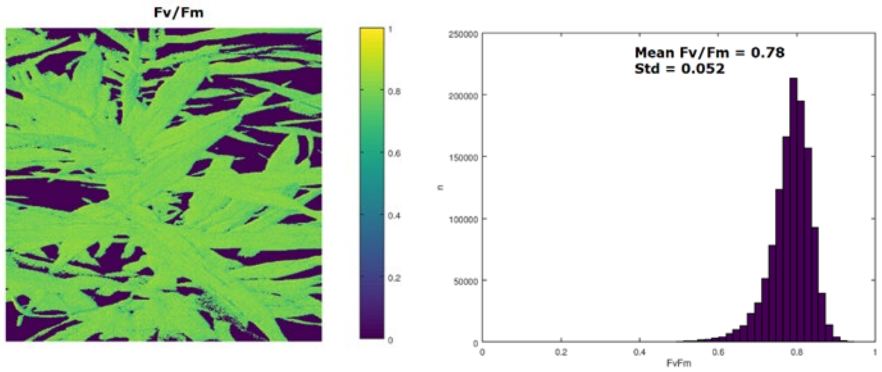
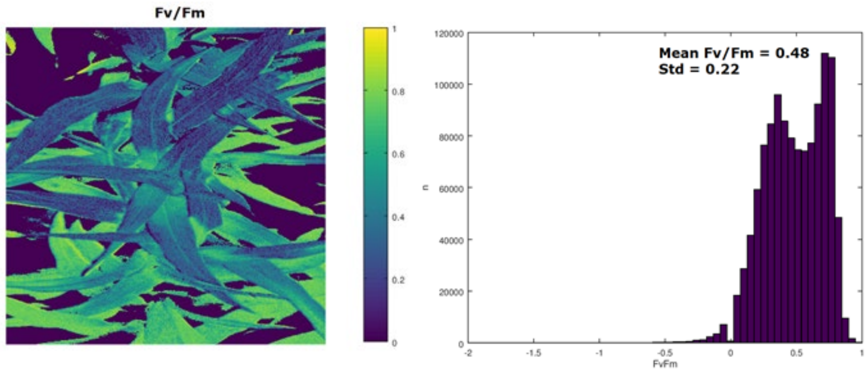


Photo credits: M Newcomb

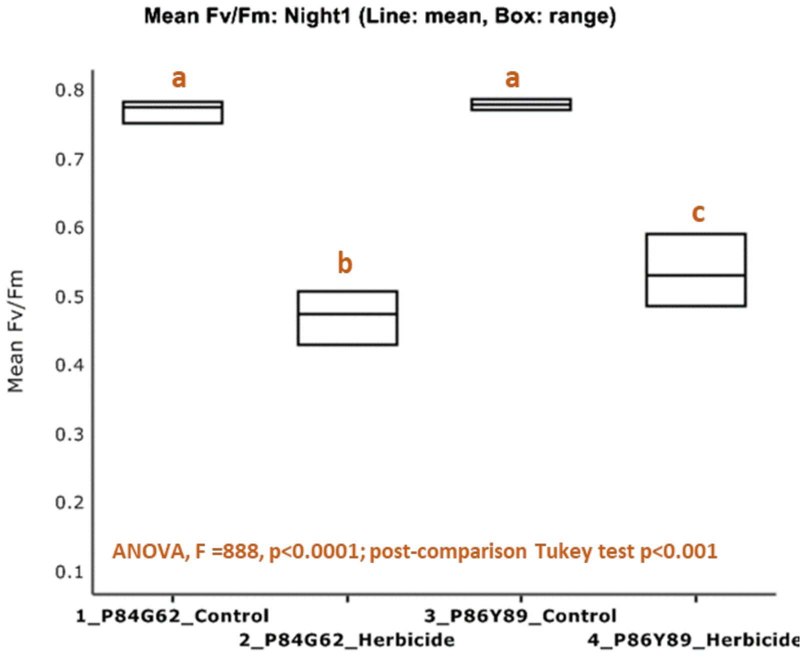
During two sorghum field trials in 2018, experiments were conducted comparing measurements on plots treated with photosystem II inhibitor herbicide (Velpar) and untreated plots. Quantum efficiency of the PSII system was estimated as Fv/Fm (normalized ratio of variable fluorescence to max fluorescence).

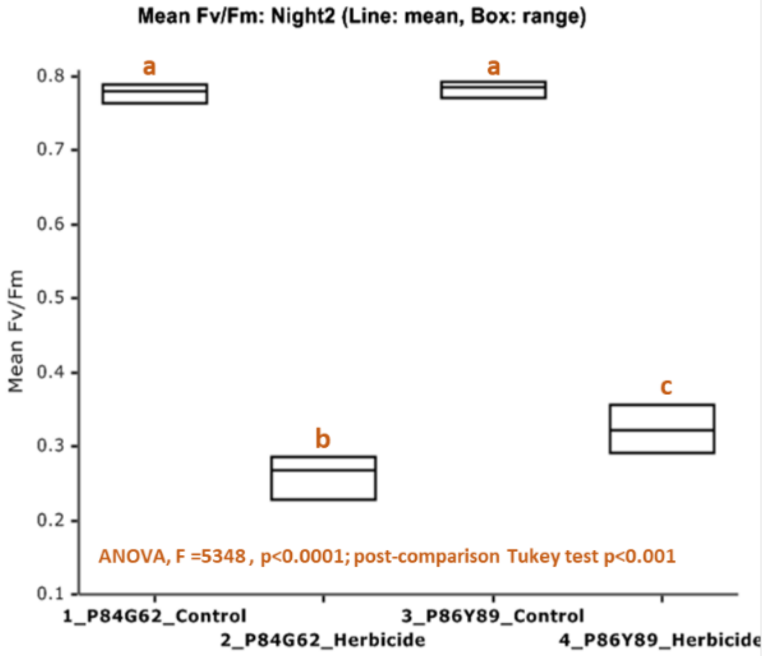


Untreated control



Treated with PSII inhibitor





October 2018 experiment, two sorghum varieties

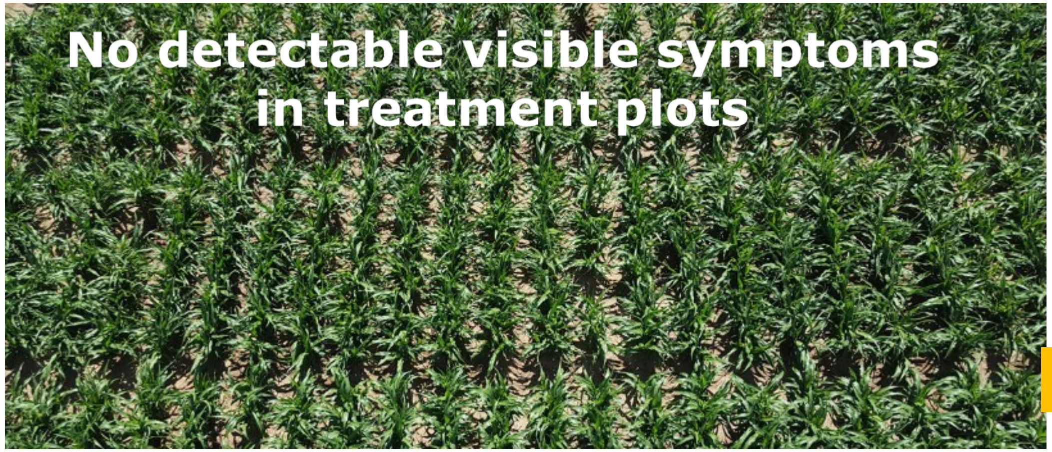


Photo credits: M Newcomb

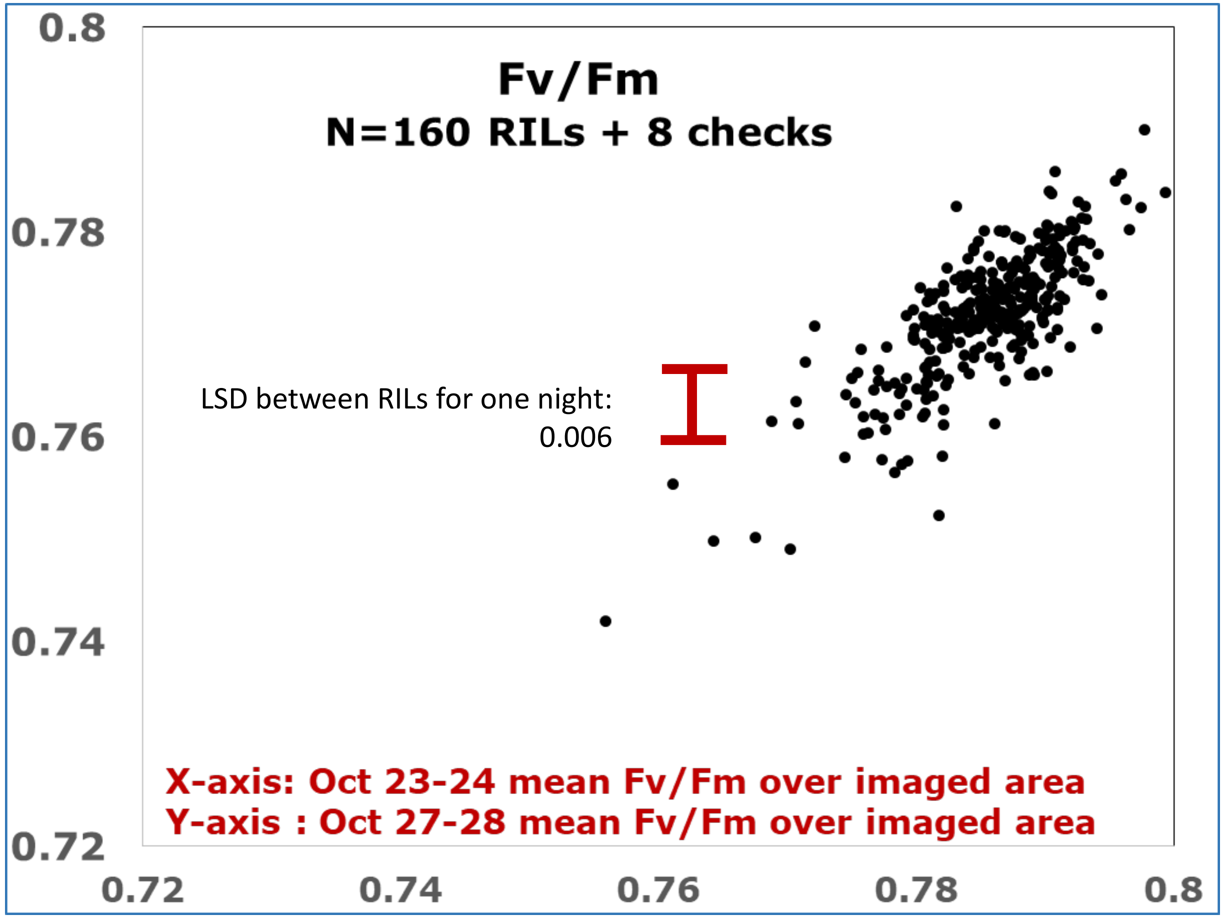
PSII- $\boldsymbol{\sigma}^2_g$

Automated Chlorophyll Fluorescence Imaging of Dark-adapted Plants in Field Plots

Maria Newcomb[1], Tino Dornbusch[2], Matthew Herritt[3], Jeffrey Demieville[1], Jeffrey W. White[3], Rick Ward[1], John Heun[1], Robert J. Strand[2], Duke Pauli[1], Nadia Shakoor[4], Todd Mockler[4]

[1]University of Arizona, Maricopa Agricultural Center, Maricopa, AZ; [2]LemnaTec, Aachen Germany and N America;
[3]USDA-ARS Arid-Land Agricultural Research Center, Maricopa, AZ; [4]Donald Danforth Plant Science Center, St. Louis, MO

Fv/Fm for 160 RILs (Tx7000 x SC56) + 8 Checks x 2 Reps x 2 nights

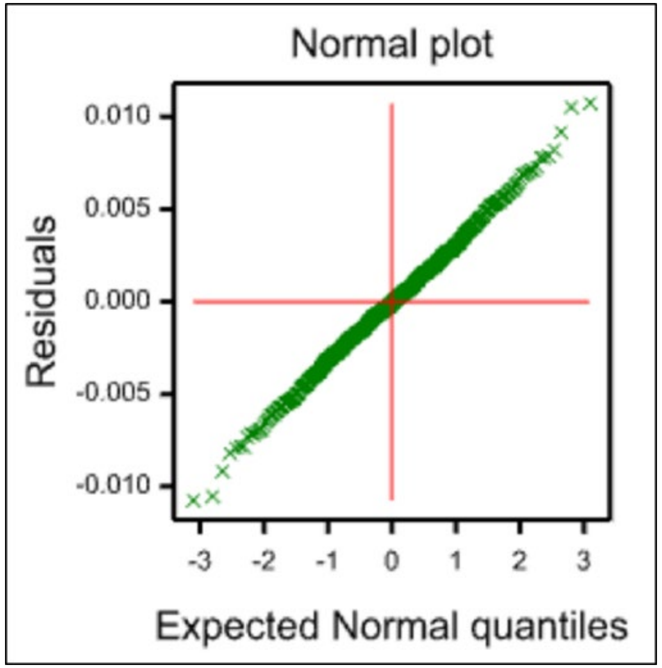


RILS provided by W Rooney (Texas A&M)

PSII in a Soghum RIL pop: Generalized Heritability >0.68

mean Fv/Fm	standard error	coefficient of variation	df
0.779	0.0046	0.6	328

	sum of squares	df	mean square	variance ratio	F pr.
Genotype	0.0138	159	0.00009	4.17	<0.001
Date	0.0296	1	0.02965	1422.46	<0.001
Genotype x date	0.0016	159	0.00001	0.49	n.s.
Residual	0.0068	328	0.00002		
Total	0.0519	647			

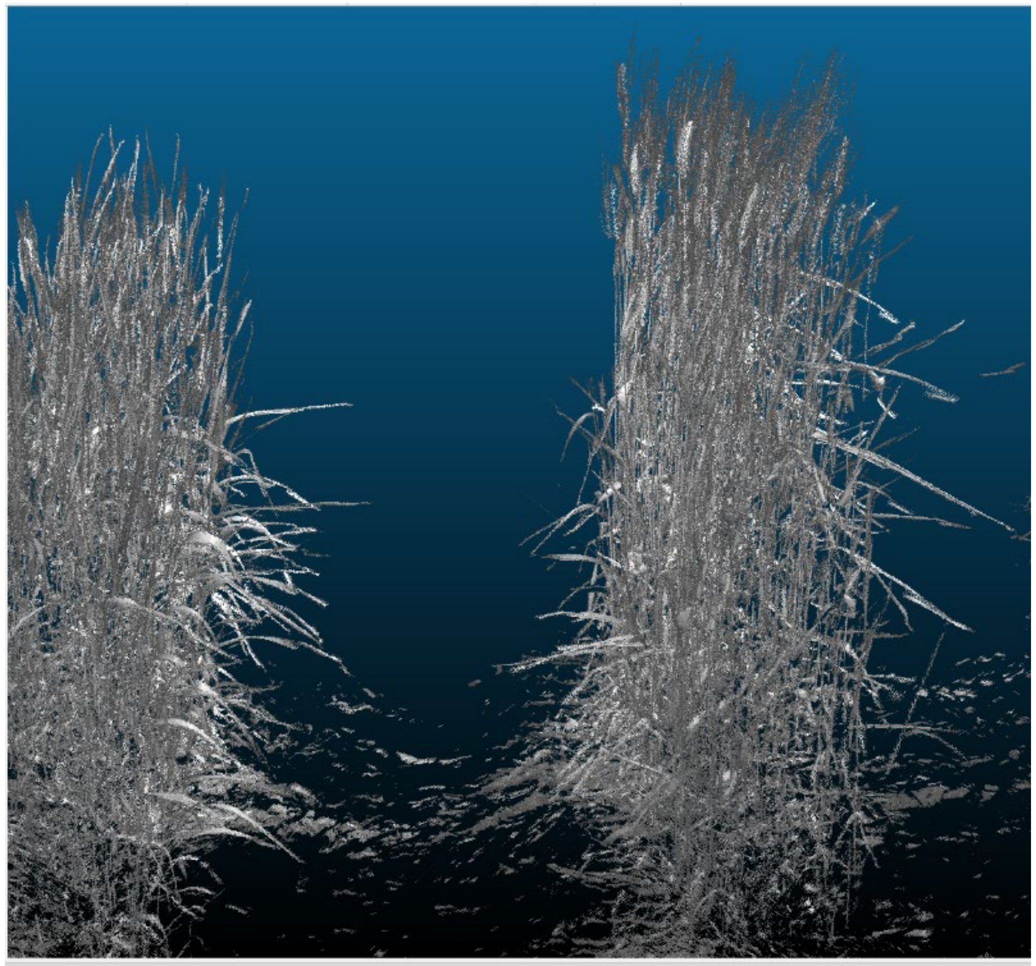


Genstat

Leaf rolling under drought: durum wheat: Fraunhofer-developed Laser Scanners

Photo credits: M Newcomb

Leaf rolling: potential for quantification from sensor-derived data products – 3D laser point clouds



TERRAREF data to be public and will/does include:

- Resequencing of over 300 of the BAP accessions
  - 9 million snps
- GBS data for the sorghum RIL
- UAV orthomosaics (thermal and multispectral) for wheat and sorghum crops (3 of wheat, 5 of sorghum)
- Gantry data for all instruments
- The Gantry data are ‘reference’ data, meant to enable optimal design of smaller systems such as those that would fit on a drone

Thanks!

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