

# Tools, resources, genotyping and phenotyping

**Clare Lister and Simon Griffiths**

**11/10/2016**

**AFM – Alba Farre Martinez**

**CL – Clare Lister**

**JZ – Ji Zhou**

**SEO – Simon Orford**

# WGIN3 Projects: Griffiths' Lab

1. Dissecting UK drought tolerance in Paragon x Garcia
2. Quantifying agronomic impact of WGIN target genes using the Paragon NIL library
3. Informing multiple marker assisted selection for yield stability using Paragon library
4. A chromosome segment substitution library for Avalon x Cadenza
5. Understanding genotype x environment interaction in Avalon x Cadenza
6. Foundations for a new generation segregating populations for studying yield stability in the UK
7. Applying WGIN data to breeding by design for UK yield stability
8. Curation and distribution of WGIN germplasm

# WGIN3 Projects: Griffiths' Lab

1. Dissecting UK drought tolerance in Paragon x Garcia **(CL)\***
2. Quantifying agronomic impact of WGIN target genes using the Paragon NIL library **In Drought and WISP Trials, and under Phenospex (CL/JZ)**
3. Informing multiple marker assisted selection for yield stability using Paragon library **Rht stacking / winter Paragon x Rht's (SEO/AFM/CL)**
4. A chromosome segment substitution library for Avalon x Cadenza **(CL)\***
5. Understanding genotype x environment interaction in Avalon x Cadenza **Publication (AFM)**
6. Foundations for a new generation segregating populations for studying yield stability in the UK **Crosses from RL (SEO)**
7. Applying WGIN data to breeding by design for UK yield stability **Crosses made and seed bulking underway (SEO/CL)**
8. Curation and distribution of WGIN germplasm **Ongoing (CL)**

## 2. Quantifying agronomic impact of WGIN target genes using the Paragon NIL library

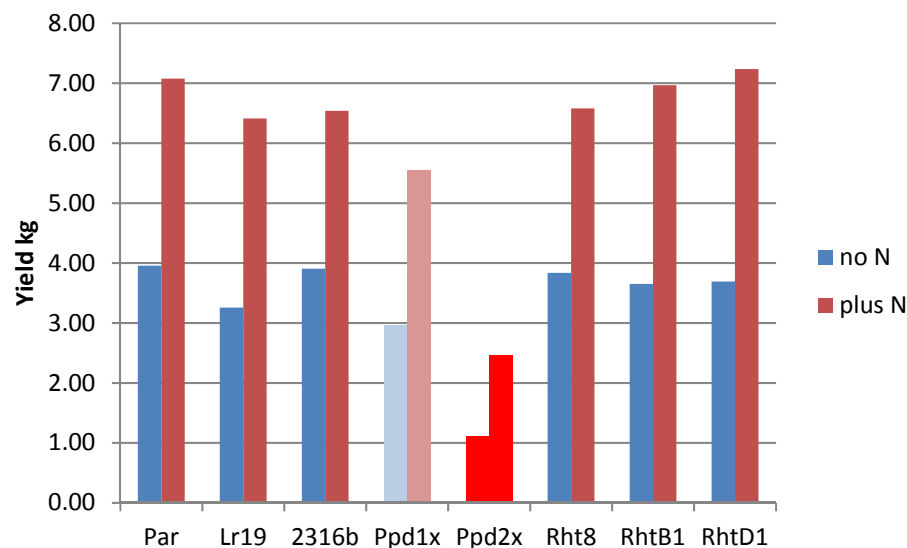
- Eight lines drilled under Phenospex
  - Two randomised blocks of 16
  - One treatment – all plots + nitrogen (40 kg/h)
  - Two subsequent treatments +/- additional nitrogen (250 kg/h)
- DTEM, Height, Yield, TGWT scored

<b>Paragon</b>
Lr19 Kamb1
Par Mutant 2316b
Ppd 1x Early
Ppd 2x Early
Rht 8 Mara
Rht B1 Robigus
Rht D1 Alchemy



## 2. Quantifying agronomic impact of WGIN target genes using the Paragon NIL library

<b>Paragon</b>
<b>Lr19 Kamb1</b>
<b>Par Mutant 2316b</b>
<b>Ppd 1x Early</b>
<b>Ppd 2x Early</b>
<b>Rht 8 Mara</b>
<b>Rht B1 Robigus</b>
<b>Rht D1 Alchemy</b>



**JIC Trial to be repeated 2016-2017**

- +/- Nitrogen
- To be drilled on land previously +/- clover

### 3. Informing multiple marker assisted selection for yield stability using Paragon library

- NIL stacking (SEO)
  - *RhtB1* x *Rht8*
  - *RhtD1* x *Rht8*
  - *RhtB1* x *RhtD1*

**Seed bulking in field  
2016-2017**



*RhtB1+RhtD1*      *Rht8+RhtD1*  
*Rht8+RhtB1*      Paragon

- Winter Paragon made by crossing in *VrnA1* + *VrnB1* from Malacca (AFM)
- Crossed into *Rht8* / *RhtB1* / *RhtD1* to produce winter, semi-dwarf, Paragon (CL/SEO)

**Seed bulking in g/h 2016-2017**

# 5. Understanding genotype x environment interaction in Avalon x Cadenza

Farré et al. *BMC Plant Biology* (2016) 16:161  
DOI 10.1186/s12870-016-0849-6


BMC Plant Biology

RESEARCH ARTICLE

Open Access



## Application of a library of near isogenic lines to understand context dependent expression of QTL for grain yield and adaptive traits in bread wheat

Alba Farré, Liz Sayers, Michelle Leverington-Waite, Richard Goram, Simon Orford, Luzie Wingen, Cathy Mumford and Simon Griffiths 

### Abstract

**Background:** Previous quantitative trait loci (QTLs) studies using the Avalon × Cadenza doubled haploid (DH) population identified eleven QTLs determining plant height, heading date and grain yield. The objectives of this study were: (i) to provide insight into the effects of these QTLs using reciprocal multiple near isogenic lines (NILs) with each pair of alleles compared in both parental backgrounds (Avalon or Cadenza), (ii) quantifying epistasis by looking at the background effects and (iii) predict favourable allelic combinations to develop superior genotypes adapted to a target environment.

**Results:** To this aim, a library of 553 BC<sub>2</sub> NILs and their recurrent parents were tested over two growing seasons (2012/2013 and 2013/2014). The results obtained in the present study validated the plant height, heading date and grain yield QTLs previously identified. Epistatic interactions were detected for the 6B QTL for plant height and heading date, 3A QTL for heading date and grain yield and 2A QTL for grain yield.

**Conclusion:** The marker assisted backcrossing strategy used provided an efficient method of resolving QTL for key agronomic traits in wheat as Mendelian factors determining possible epistatic interactions. The study shows that these QTLs are amenable to marker assisted selection, fine mapping, future positional cloning, and physiological trait dissection.

**Keywords:** Yield, Wheat, Near isogenic lines, QTL

## 6. Foundations for a new generation segregating populations for studying yield stability in the UK

Female		Male	RL value Diversity target	RL value Diversity target	RL value Diversity target	DTEM HT LODGE GS31 YLD
Grafton	x	Conqueror	lodging	mildew	eyespot	y
Grafton	x	Revelation	heading	brown rust	fusarium	y
Beluga	x	Cordiale	protein	hagberg	TGW	
Scout	x	Denman	lodging			y
Revelation	x	Gallant	heading	fusarium		y
KWS Sterling	x	Alchemy	height			y
KWS Santiago	x	Scout	yield	eyespot		y
KWS Santiago	x	Solstice	yield			y
KWS Santiago	x	Gallant	hagberg	market share		
KWS Kielder	x	Scout	yield			y
KWS Kielder	x	Einstein	yield			y
KWS Gator	x	Gallant				
KWS Gator	x	Revelation	septoria tritici			
KWS Croft	x	Scout	lodging			y
Icon	x	Skyfall				
Horatio	x	KWS Gator	GS31			y
Grafton	x	Solstice	height	eyespot		y
Gallant	x	Invicta	heading	market share		y
Gallant	x	KWS Kielder	market share			
Einstein	x	KWS Santiago	yield			y
Cougar	x	Claire	yield untr			y
Cordiale	x	Revelation	heading	brown rust		y
Cordiale	x	Crusoe	GS31	market share		y
Cordiale	x	Alchemy	height			y
Cordiale	x	Invicta	heading			y
Conqueror	x	Scout	lodging	eyespot		y
Claire	x	Revelation	yield untr			y

- All crosses at F2
- Populations in yellow currently being taken forward (SEO)



# 7. Applying WGIN data to breeding by design for UK yield stability

Mol Breeding (2015) 35:70  
DOI 10.1007/s11032-015-0268-7

## Using the UK reference population Avalon × Cadenza as a platform to compare breeding strategies in elite Western European bread wheat

Juan Ma · Luzie U. Wingen · Simon Orford ·  
Paul Fenwick · Jiankang Wang · Simon Griffiths

Received: 9 May 2014 / Accepted: 15 September 2014  
© The Author(s) 2015. This article is published with open access at Springerlink.com

**Abstract** Wheat breeders select for qualitative and quantitative traits, the latter often detected as quantitative trait loci (QTL). It is, however, a long procedure from QTL discovery to the successful introduction of favourable alleles into new elite varieties and finally into farmers' crops. As a proof of principle for this process, QTL for grain yield (GY), yield components, plant height (PH), ear emergence (EM), solid stem (SS) and yellow rust resistance (*Yr*) were identified in segregating UK bread wheat reference population, Avalon × Cadenza. Among the 163 detected QTL were several not reported before: 17 for GY, the major GY QTL on 2D; a major SS QTL on 3B; and *Yr6 on 7B*. Common QTL were identified on ten chromosomes,

most interestingly, grain number (GN) was found to be associated with *Rht-D1b*; and GY and GN with a potential new allele of *Rht8*. The interaction of other QTL with GY and yield components was discussed in the context of designing a UK breeding target genotype. Desirable characteristics would be: similar PH and EM to Avalon; *Rht-D1b* and *Vrn-A1b* alleles; high TGW and GN; long and wide grains; a large root system, resistance to diseases; and maximum GY. The potential of the identified QTL maximising transgressive segregation to produce a high-yielding and resilient genotype was demonstrated by simulation. Moreover, simulating breeding strategies with F<sub>2</sub> enrichment revealed that the F<sub>2</sub>-DH procedure was superior to the RIL and the modified SSD procedure to achieve that genotype. The proposed strategies of parent selection and breeding methodology can be used as guidance for marker-assisted wheat breeding.

**Electronic supplementary material** The online version of this article (doi:10.1007/s11032-015-0268-7) contains supplementary material, which is available to authorized users.

## 7. Applying WGIN data to breeding by design for UK yield stability

- ‘Ideal’ DH lines to be crossed selected by analysis of the QTL data
- These would have three high yielding QTL (GY) alleles
- However most ‘ideal’ lines had unfavourable QTL alleles elsewhere
- Therefore made best selection possible...

Cross	Parent 1	Parent 2	
<b>Cross 1</b>	DH109	DH160	<b>DH109</b> is high yielding parent. <b>DH160</b> has complementary alleles at all selected loci for achieving target genotype
<b>Cross 2</b>	DH61	DH182	Neither parents carried all favourable GY alleles, but between the parents all favourable alleles were present.
<b>Cross 3</b>	DH27	DH61	Neither parents carried all favourable GY alleles, but between the parents all favourable alleles were present.

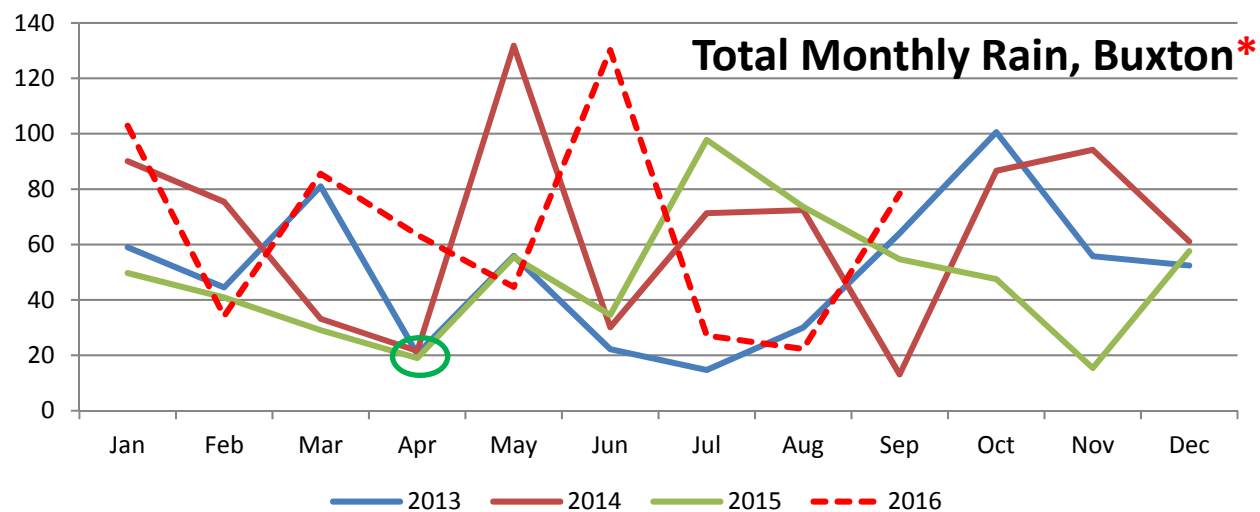
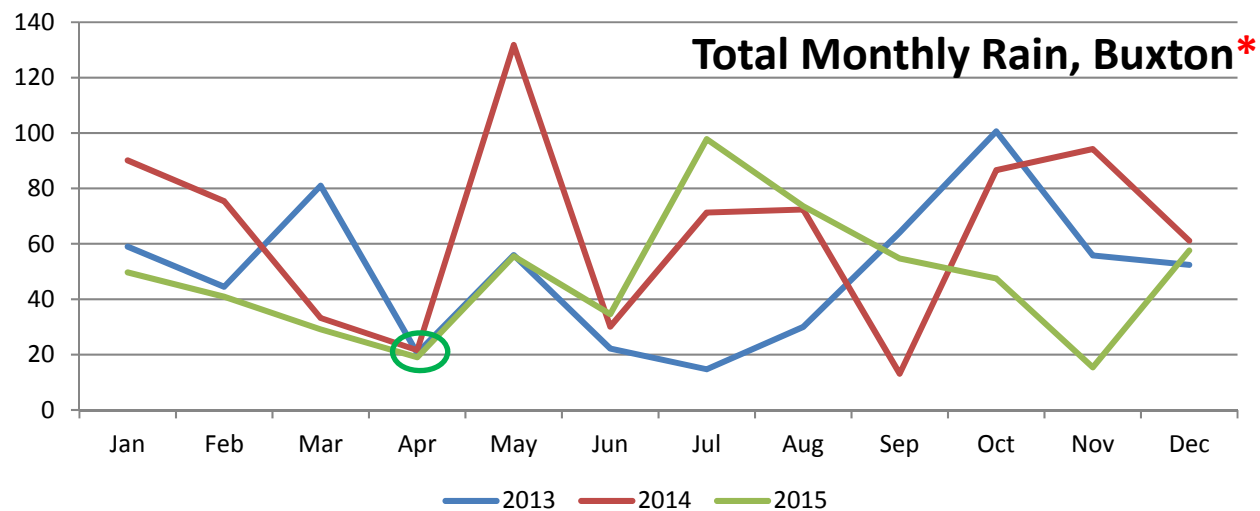
- F2 seed being generated but few F1 seed...

# 1. UK drought tolerance in Paragon x Garcia

- Paragon x Garcia (PxG) RIL population produced within WGIN to specifically target UK drought
- Paragon (UK spring wheat) x Garcia (grown in southern France & northern Spain, adapted to drought stress).
- Looking for drought-tolerant characteristics in non-UK wheat varieties highly advantageous -> breeding programme



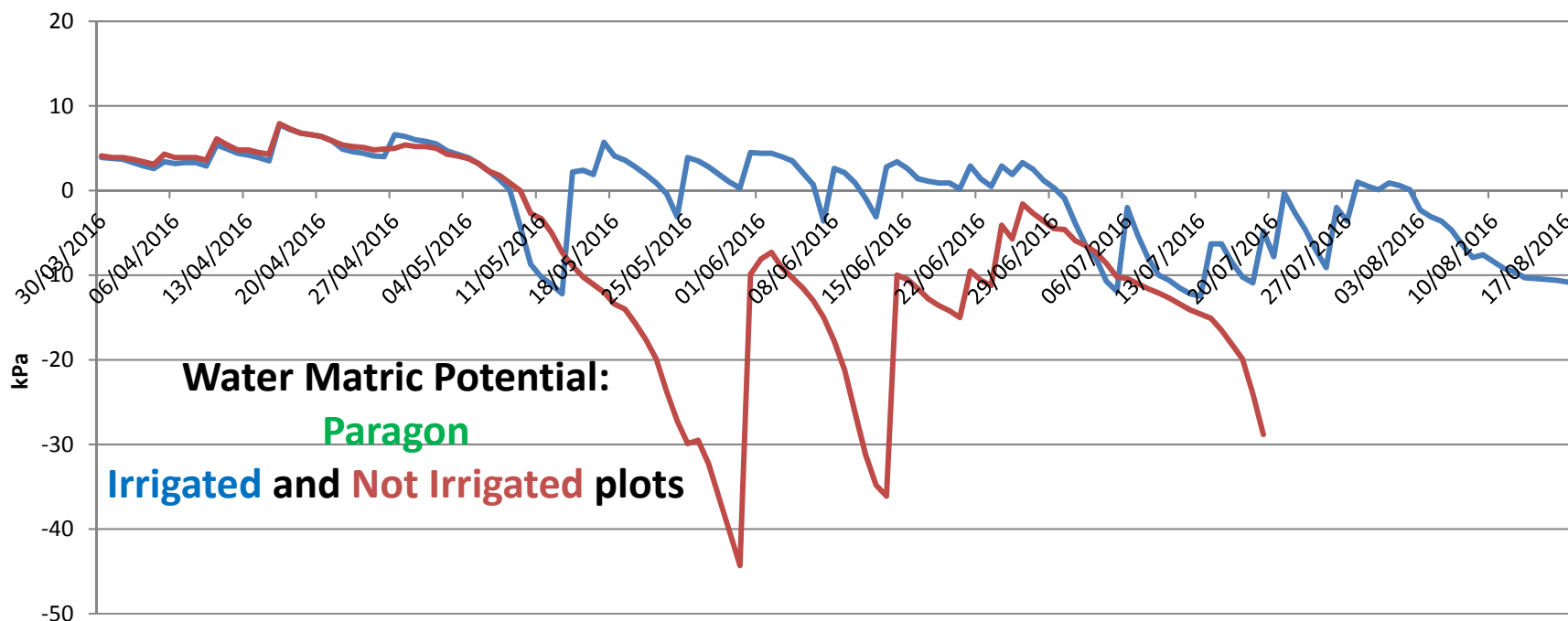
# 1. UK drought tolerance in Paragon x Garcia



Weather data courtesy of <http://www.buxton-weather.co.uk/weather.htm> \* Buxton is 13 miles from the John Innes Centre

# 1. UK drought tolerance in Paragon x Garcia

- Once weather drier used irrigation to *maintain* a water matric potential (kPa) difference between the plots



# 1. UK drought tolerance in Paragon x Garcia



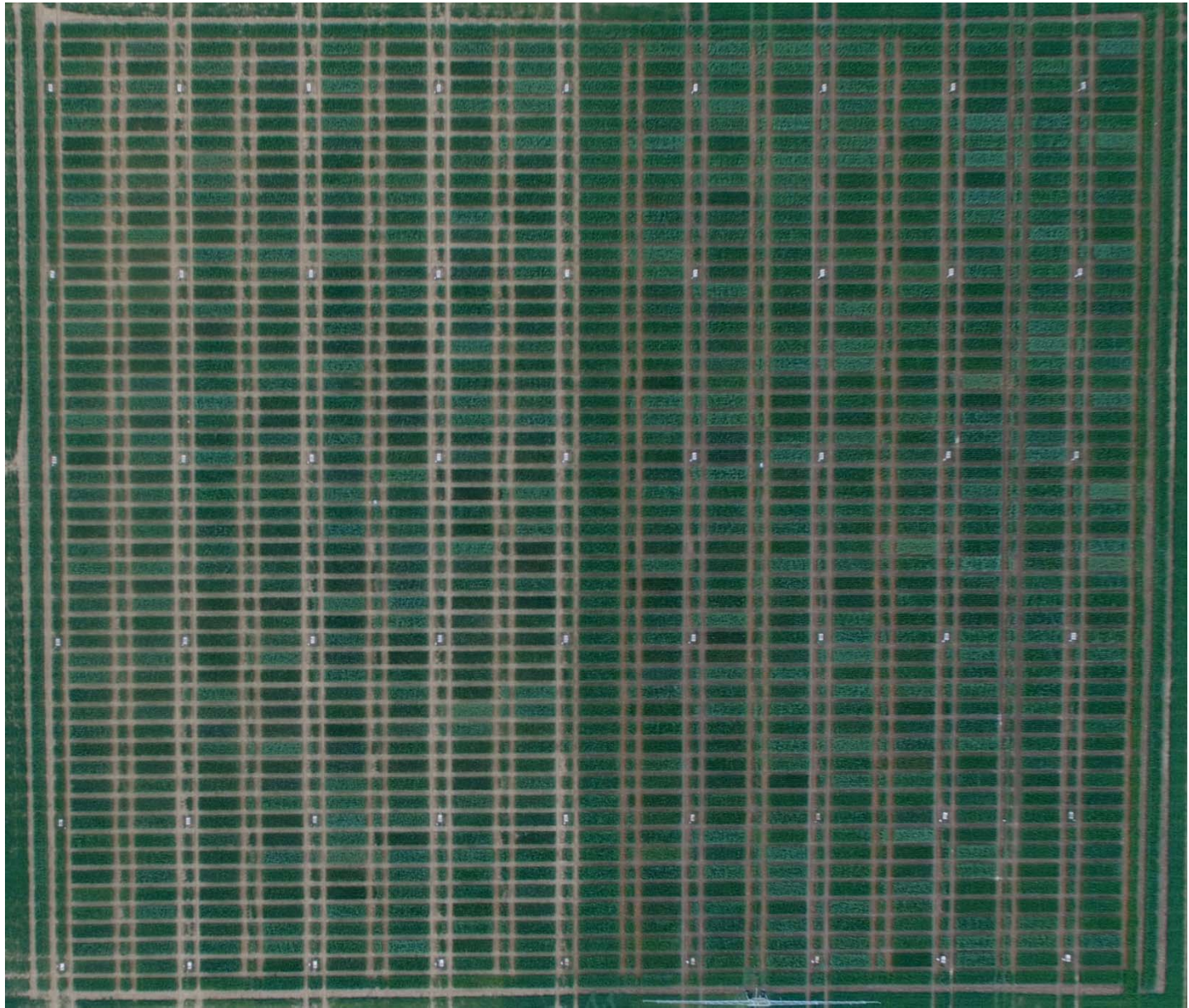
- Measured stage 31 (P x Gar)
- Stem sections around stage 31 – 1 rep only
- Measured Booting , DTEM and Height + *QTL mapping*
- Measured Yield + *QTL mapping*
- Measured TGWT and Specific Weight (1 rep so far) + *QTL mapping*
- Photos of each plot – 1 rep only
- Periodic imaging of plots during growth to track senescence, using UAV (SEO)

# 1. UK drought tolerance in Paragon x Garcia

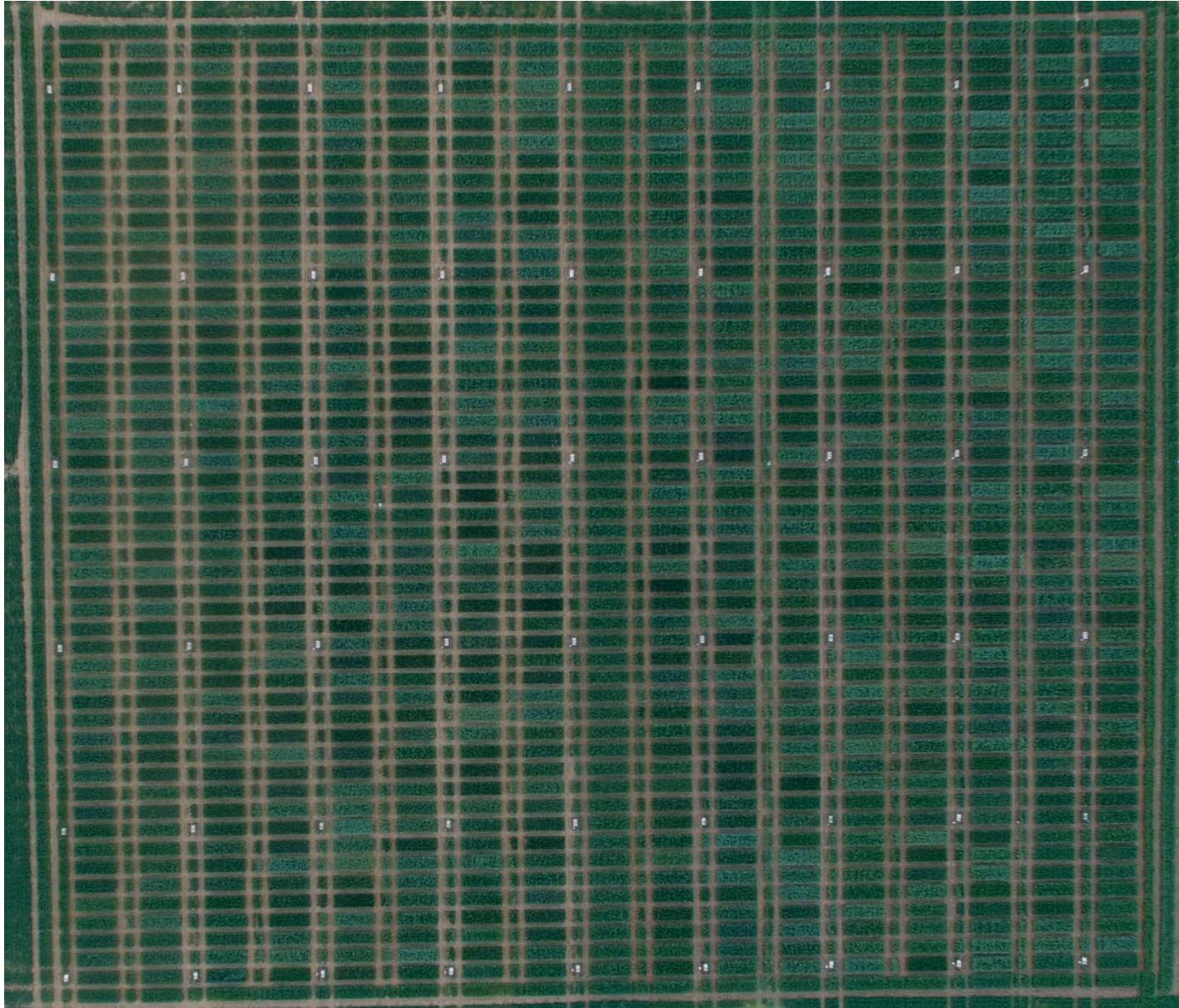
NOT IRRIGATED REPS

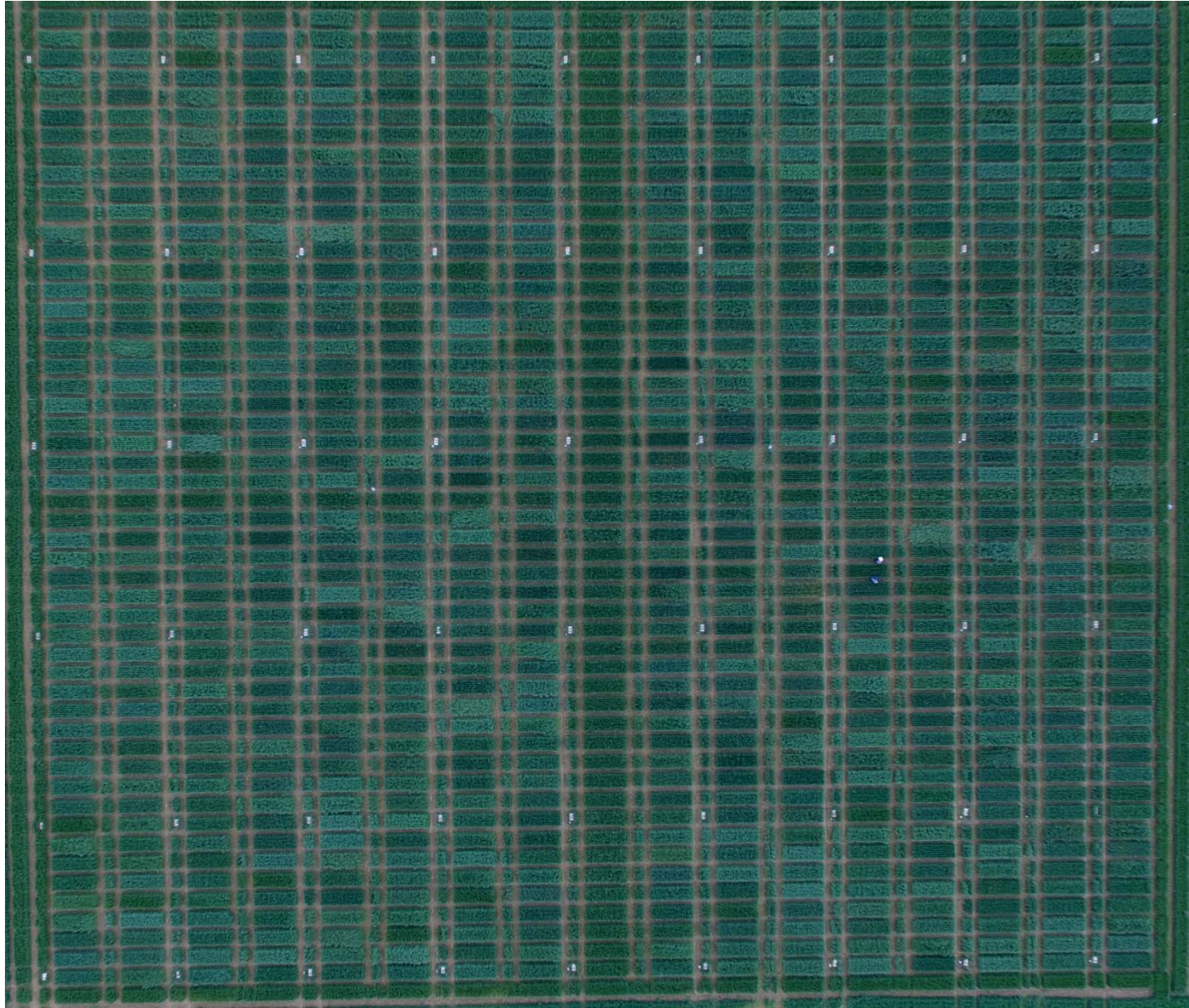


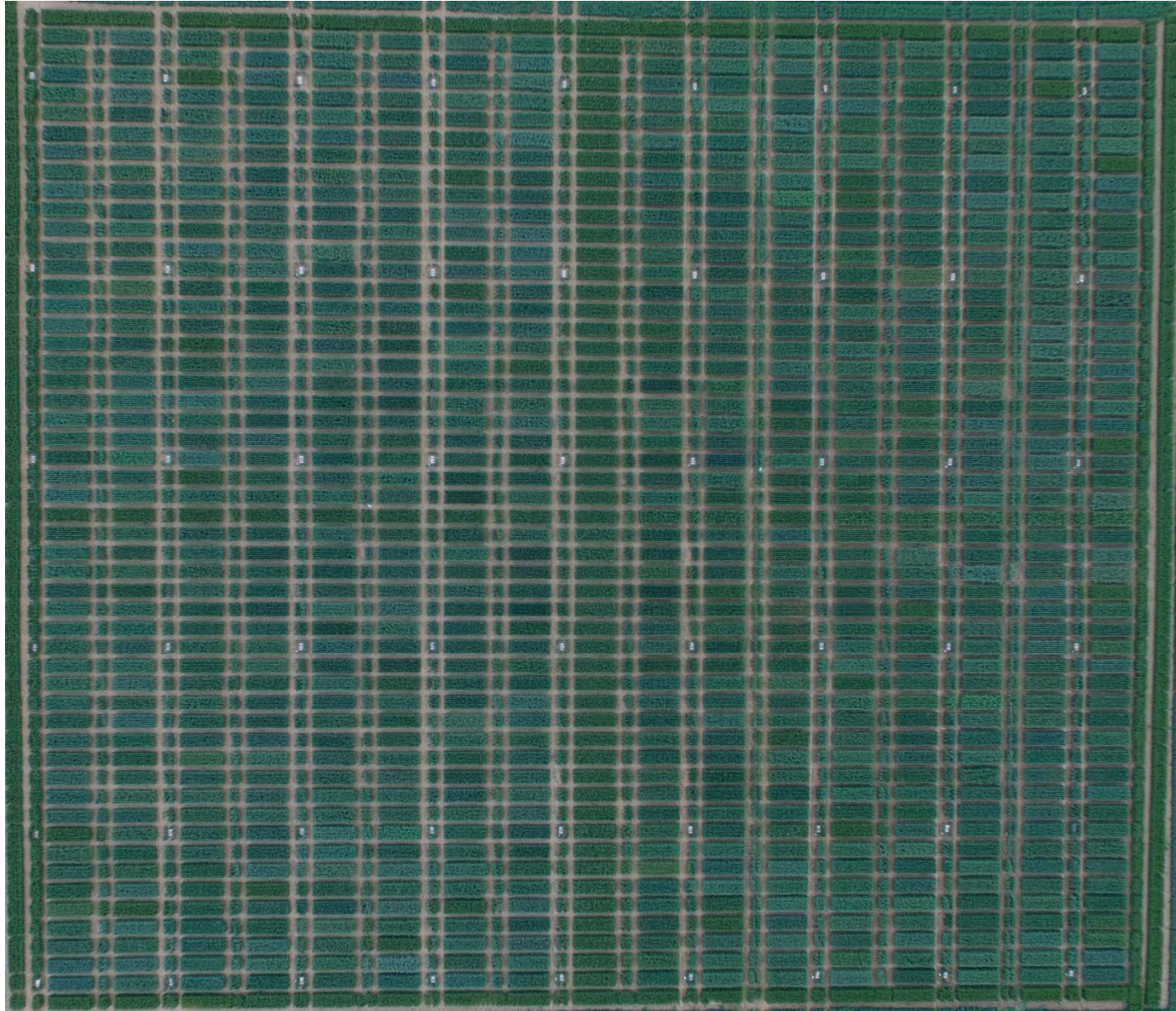
IRRIGATED REPS

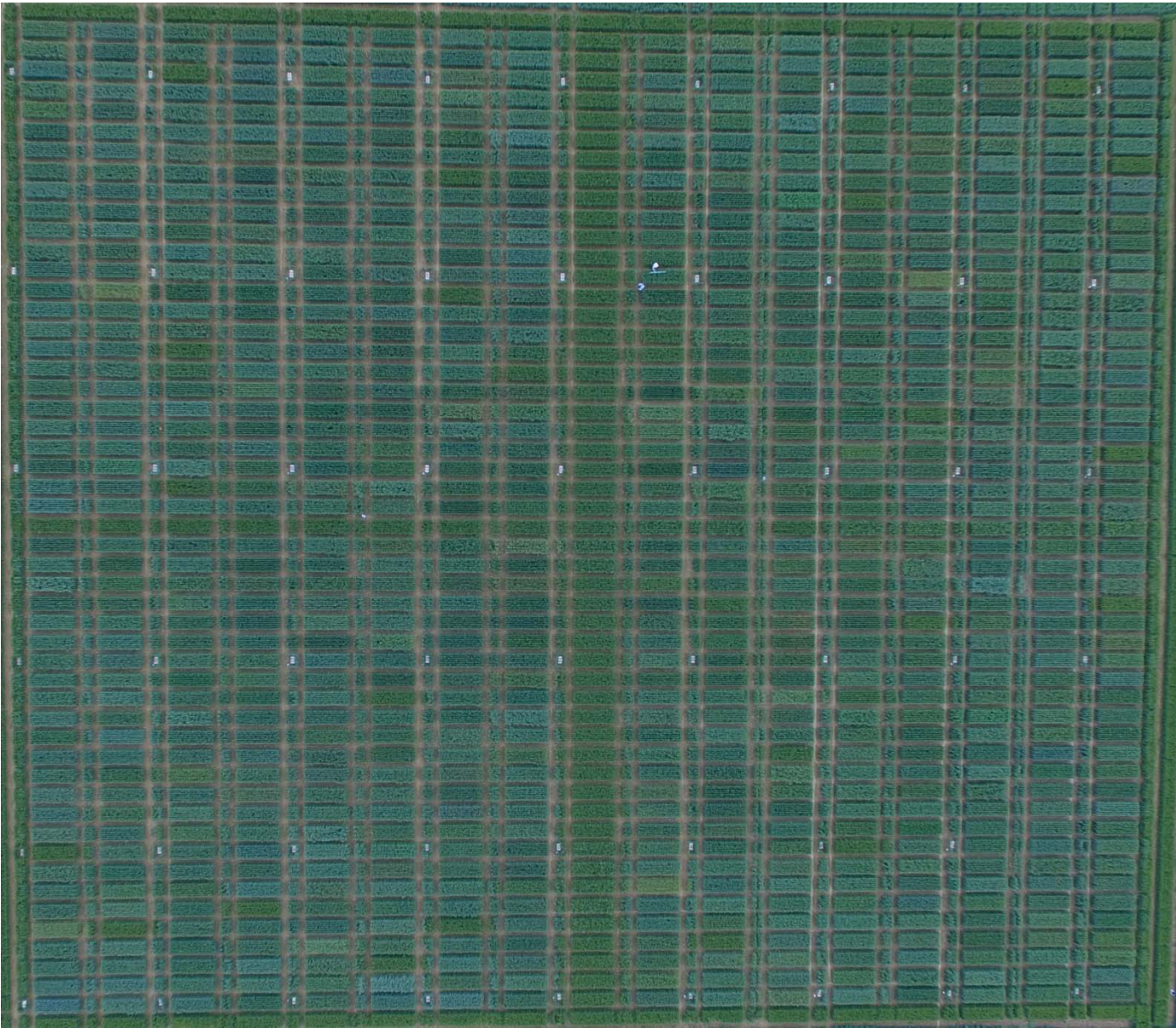


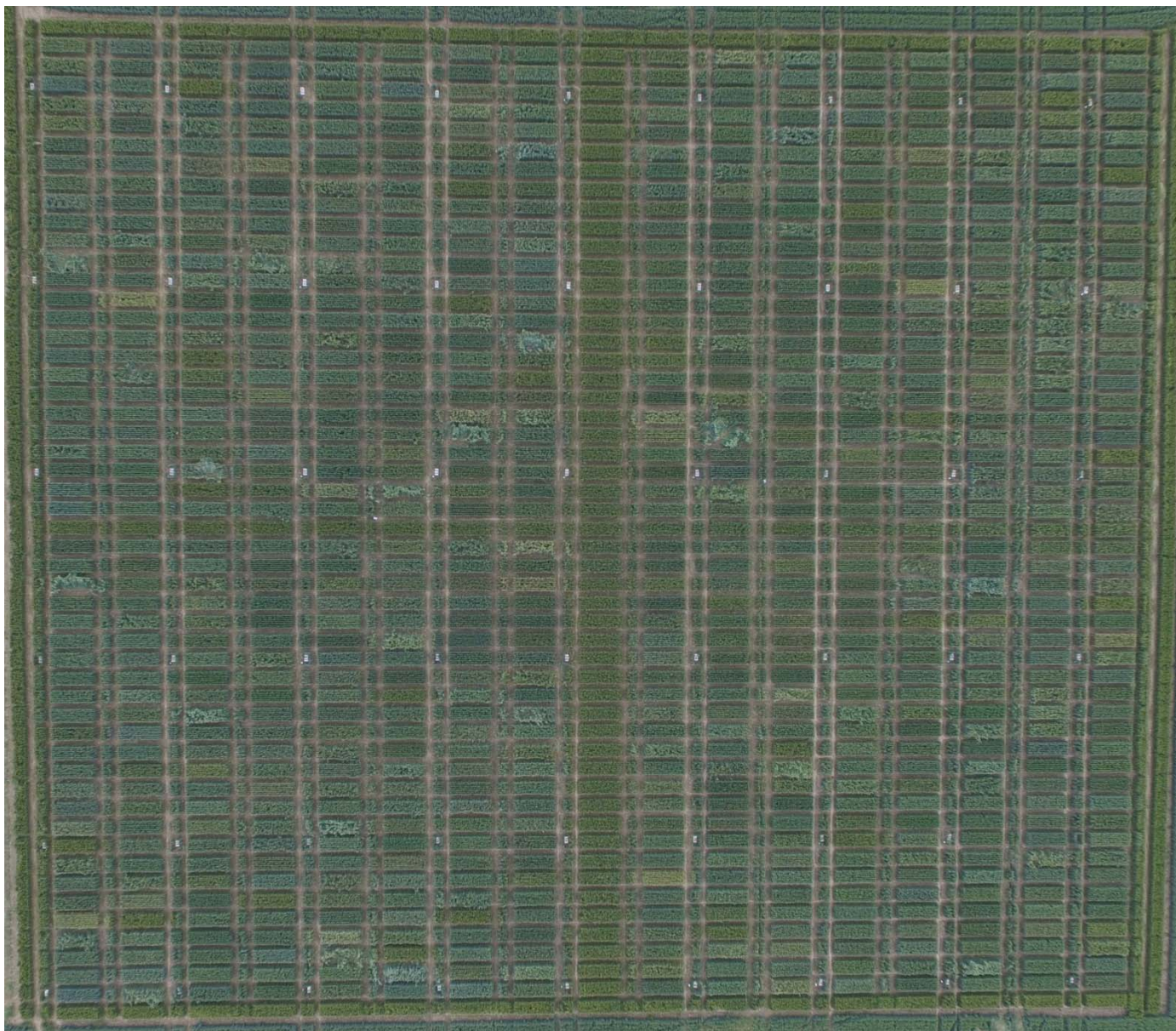




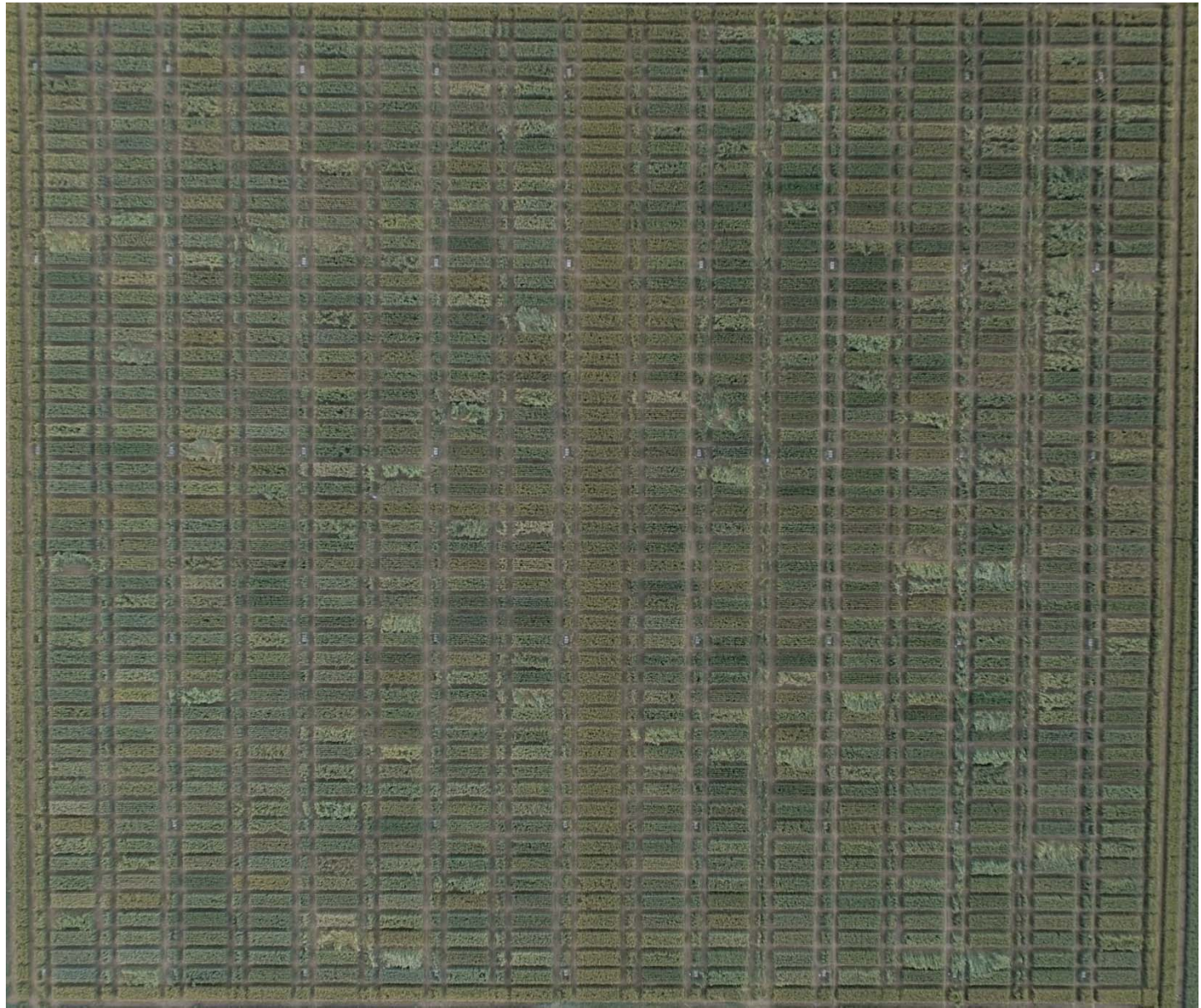












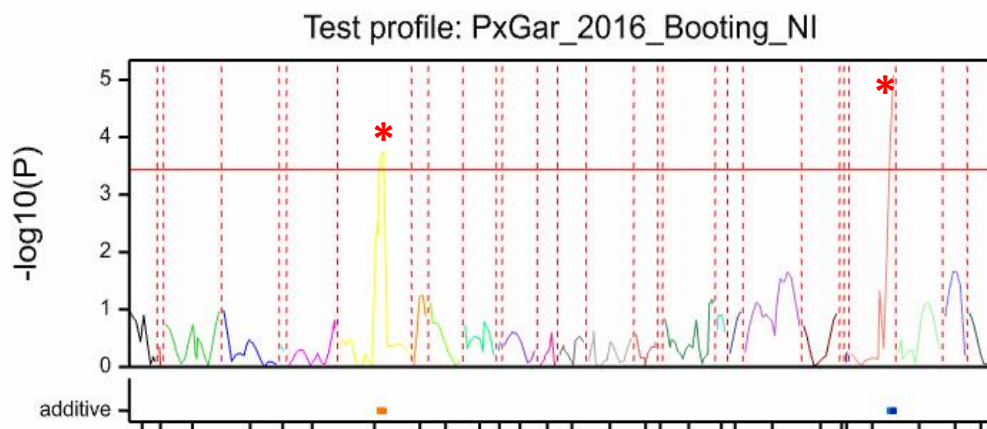




- 
- Possibility of using digital assignment of colour to monitor senescence

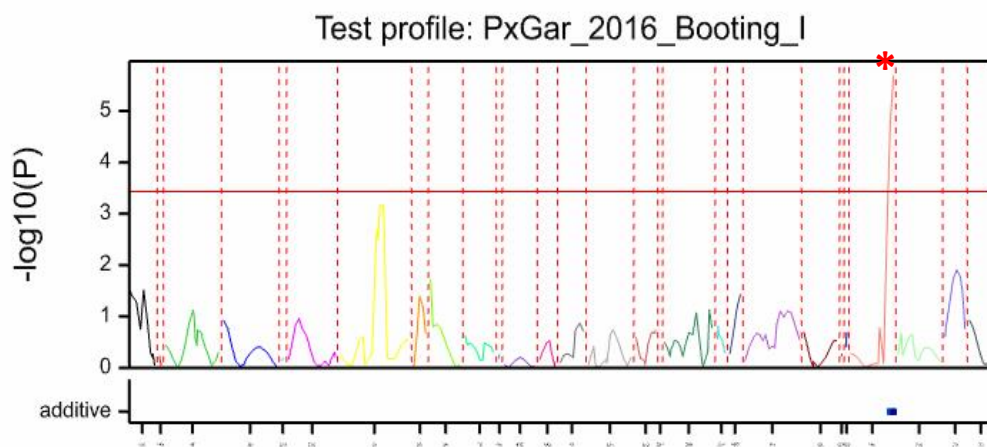
- Score and map lodging

# 1. UK drought tolerance in Paragon x Garcia



QTL mapping: **Booting**

**Booting\_NI** = QTL on 2B and 7A



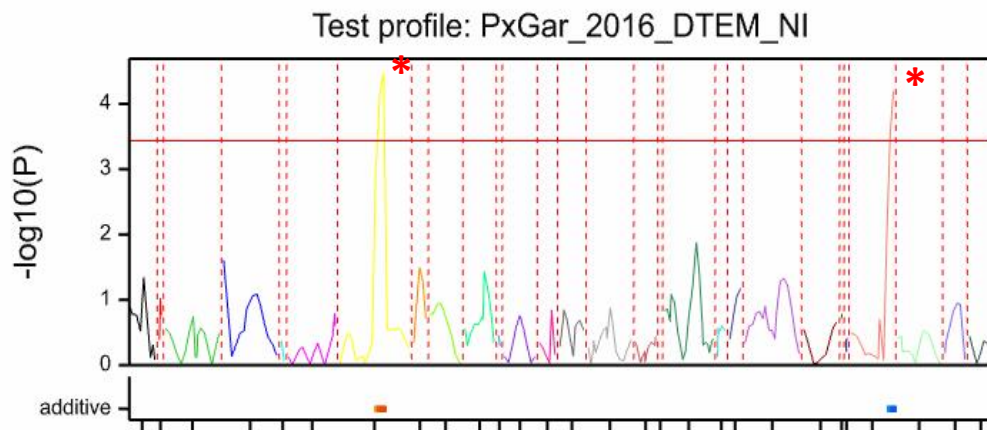
**Booting\_I** = QTL only on 7A

Chromosomes

QTL effects:

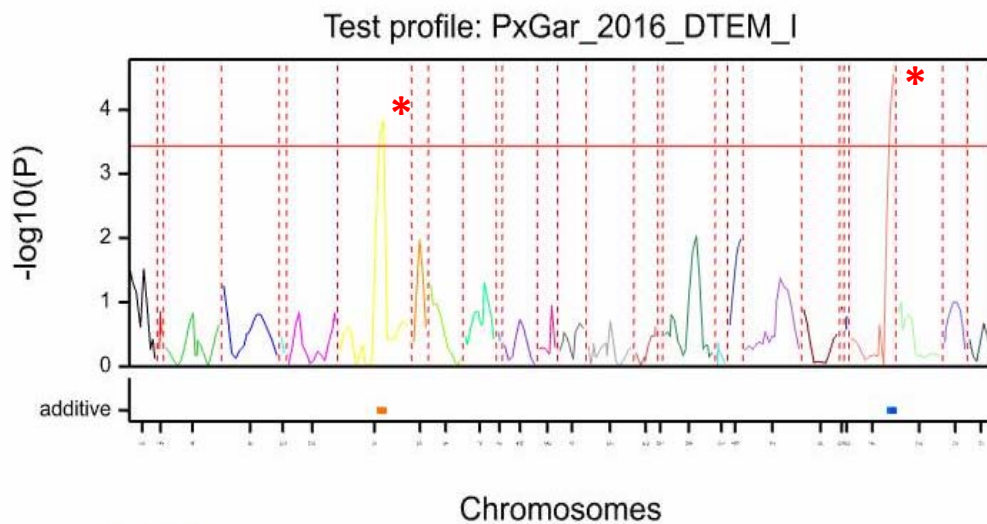
additive (blue=Parent 1 ; yellow-red=Parent 2)

# 1. UK drought tolerance in Paragon x Garcia



QTL mapping: **DTEM**

**DTEM\_NI** = QTL on 2B and 7A

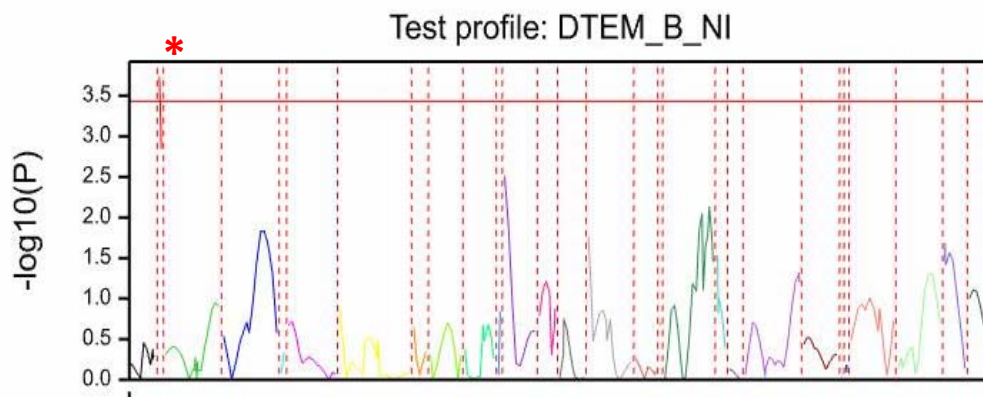


**DTEM\_I** = QTL on 2B and 7A

QTL effects:  
additive (blue=Parent 1 ; yellow-red=Parent 2)

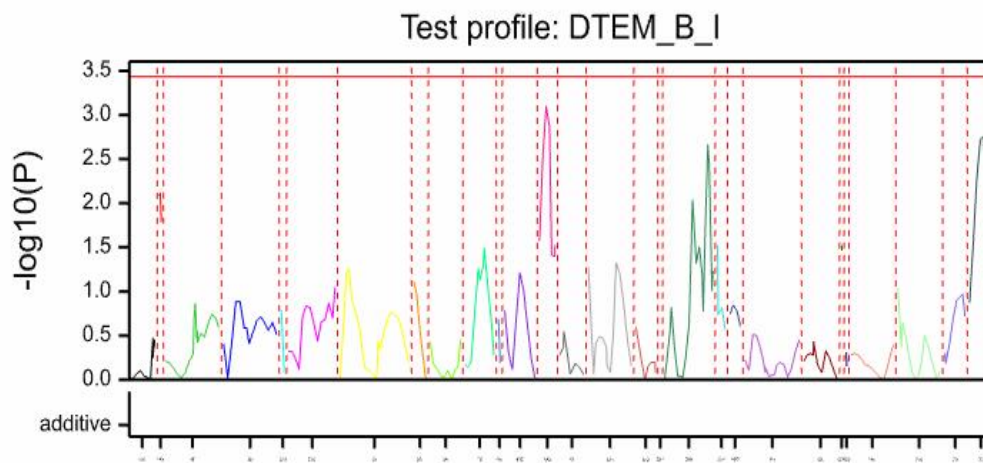
**2B and 2D (*PpdD1*?) QTL in full PxG RILs, 2015**

# 1. UK drought tolerance in Paragon x Garcia



QTL mapping: **DTEM-Booting**

DTEM-B\_NI = QTL on 1A



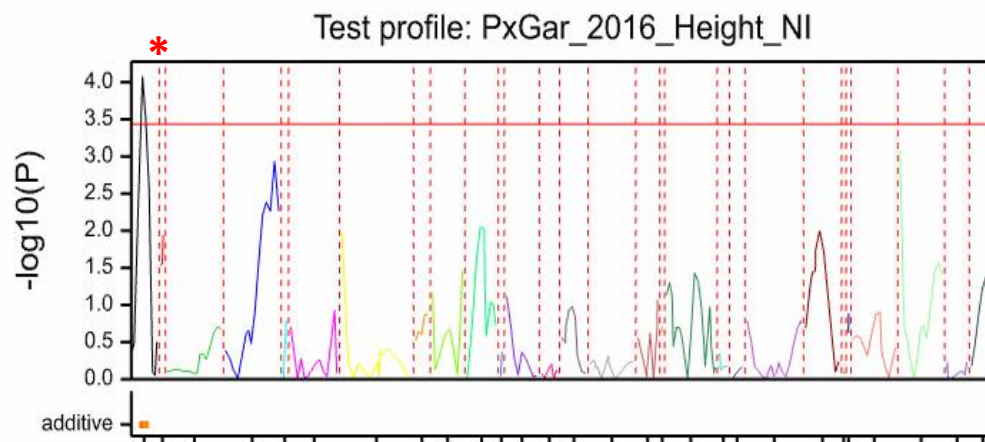
DTEM-B\_I = **NO QTL**

Chromosomes

QTL effects:

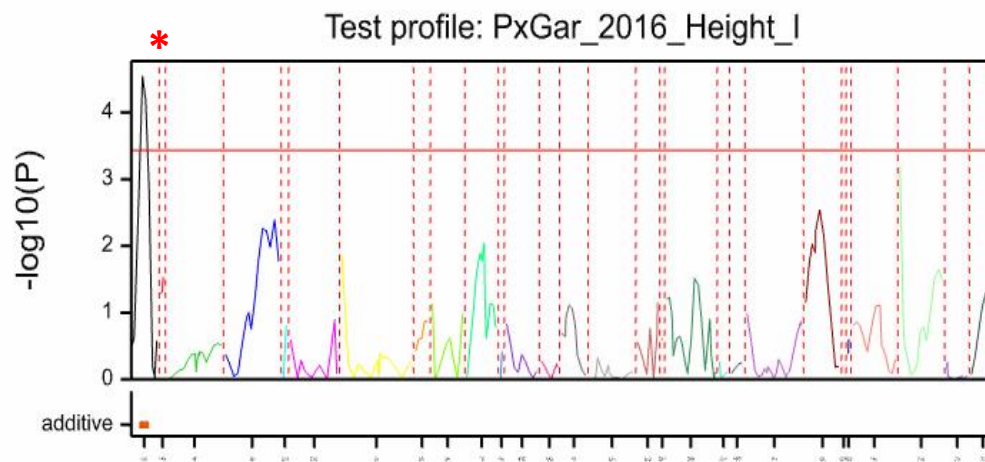
additive (blue=Parent 1 ; yellow-red=Parent 2)

# 1. UK drought tolerance in Paragon x Garcia



QTL mapping: **Height (manual)**

Height\_NI = QTL on 1A



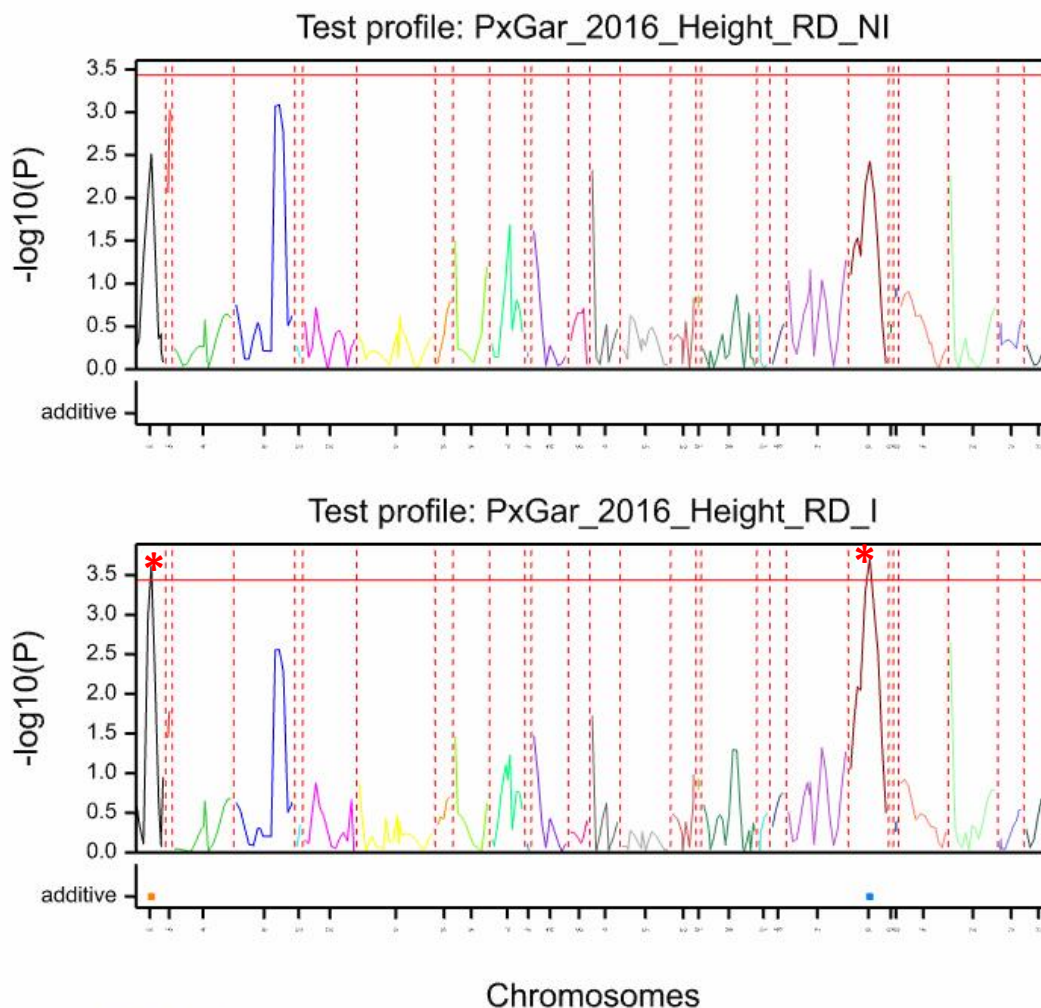
Height\_I = QTL on 1A

Chromosomes

QTL effects:  
additive (blue=Parent 1 ; yellow-red=Parent 2)

2D (*PpdD1*?) QTL in full PxG RILs, 2015

# 1. UK drought tolerance in Paragon x Garcia



QTL effects:  
additive (blue=Parent 1 ; yellow-red=Parent 2)

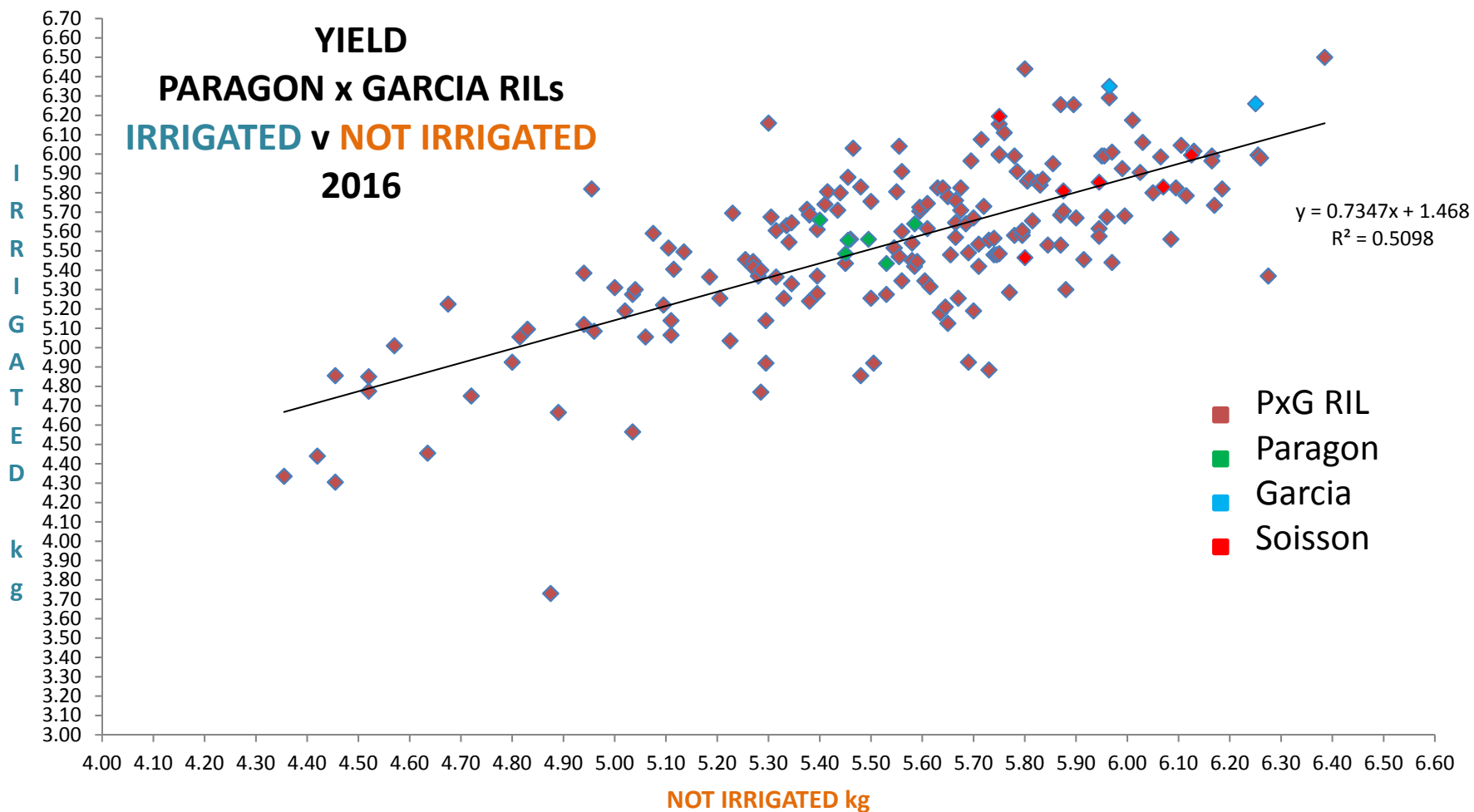
QTL mapping: **Height**  
**(Roth Drone)**

Height\_NI = No QTL  
Plants bent over more than  
irrigated plants?

Height\_I = QTL on 1A and 6B

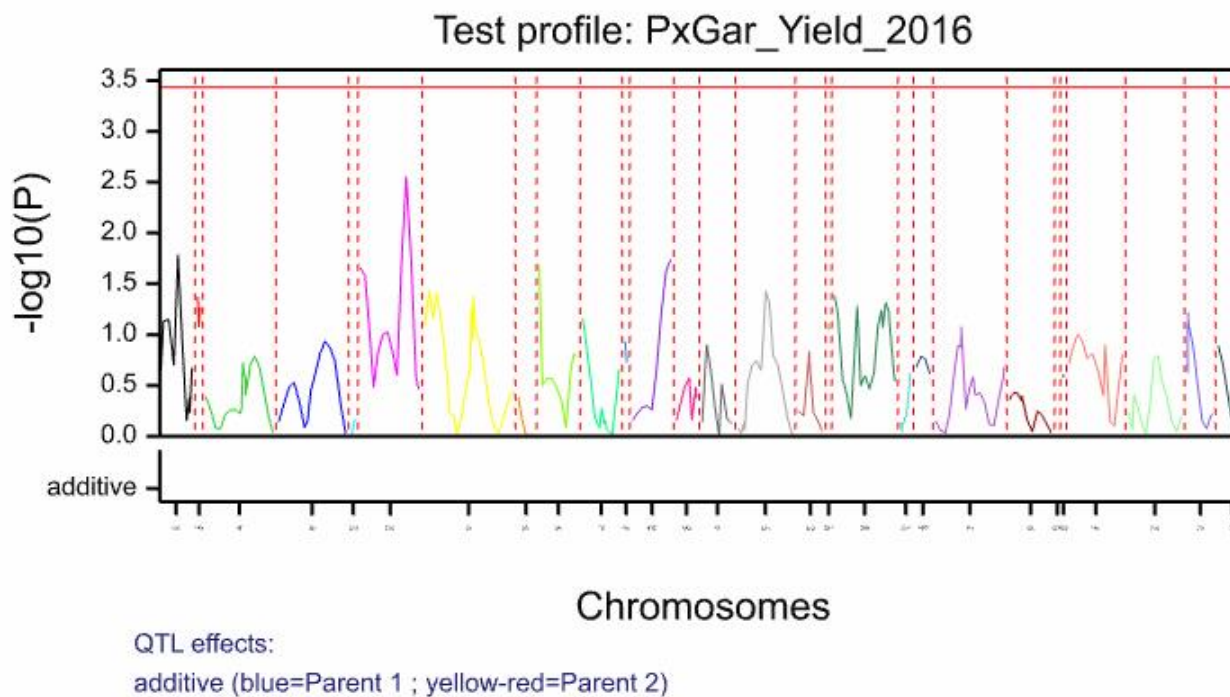
Same 1A QTL as seen in manual  
height measurements

# 1. UK drought tolerance in Paragon x Garcia



Irrigated = 4.4 kg / Not Irrigated = 4.0 kg

# 1. UK drought tolerance in Paragon x Garcia



- No QTLs identified for Yield for mean of ALL Yield data /NI and I individual data/I-NI data.
- Yield QTL previously identified using **all** PxGar RILs

2B, 2D and 7A QTL in full PxG RILs, 2015

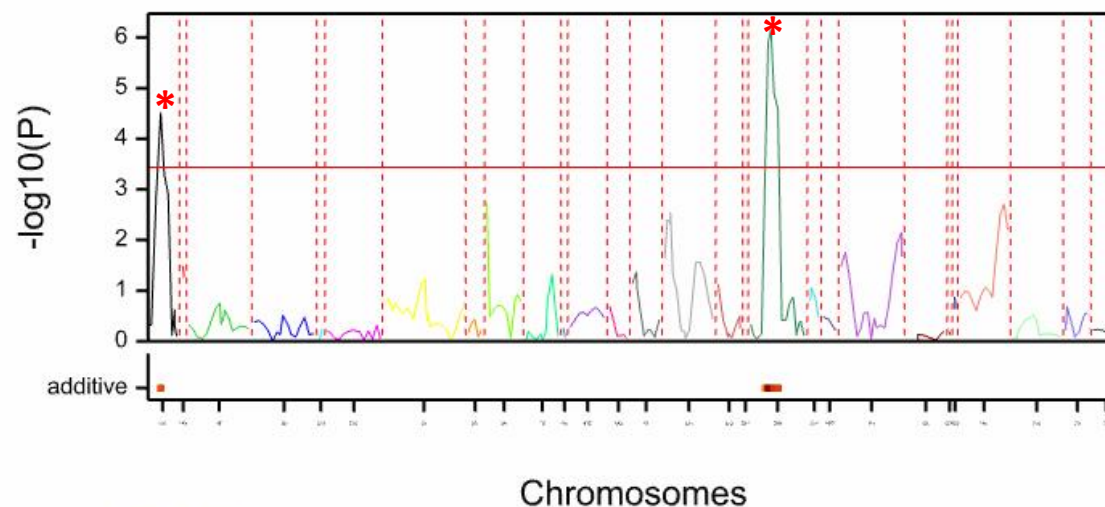


# 1. UK drought tolerance in Paragon x Garcia

## QTL mapping: TGWT

TGWT\_NI (data not available yet)

Test profile: PxGar\_TGWT\_2016 Single irrigated plot

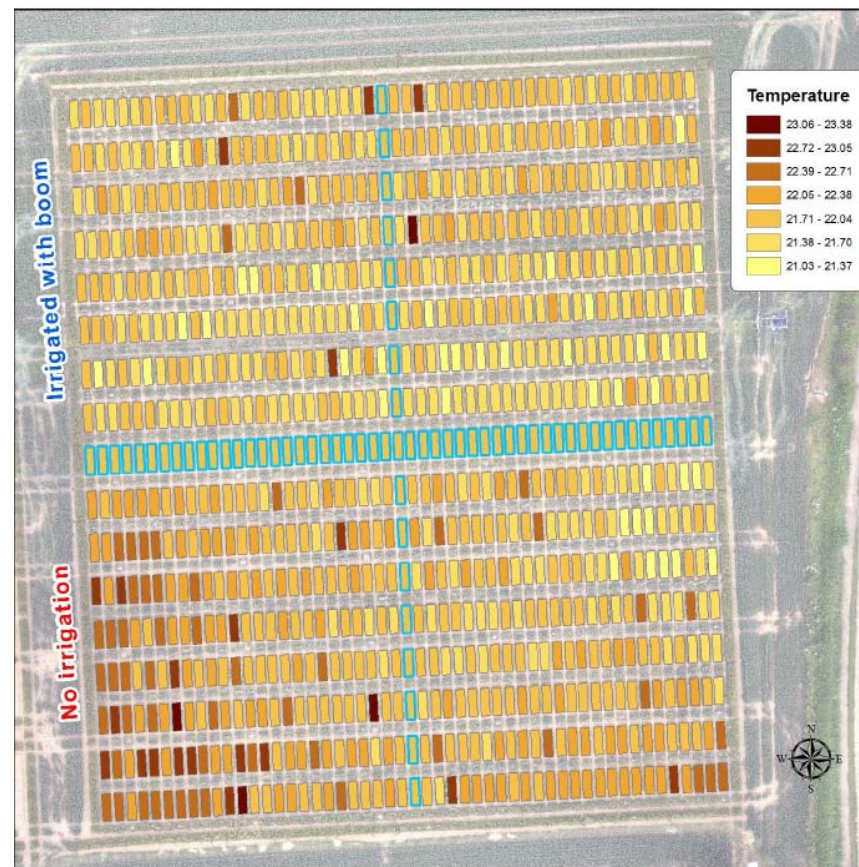


TGWT\_I = QTL on 1A and 5B

QTL effects:  
additive (blue=Parent 1 ; yellow-red=Parent 2)

1A, 2B and 5B QTL in same PxG RILs, 2015

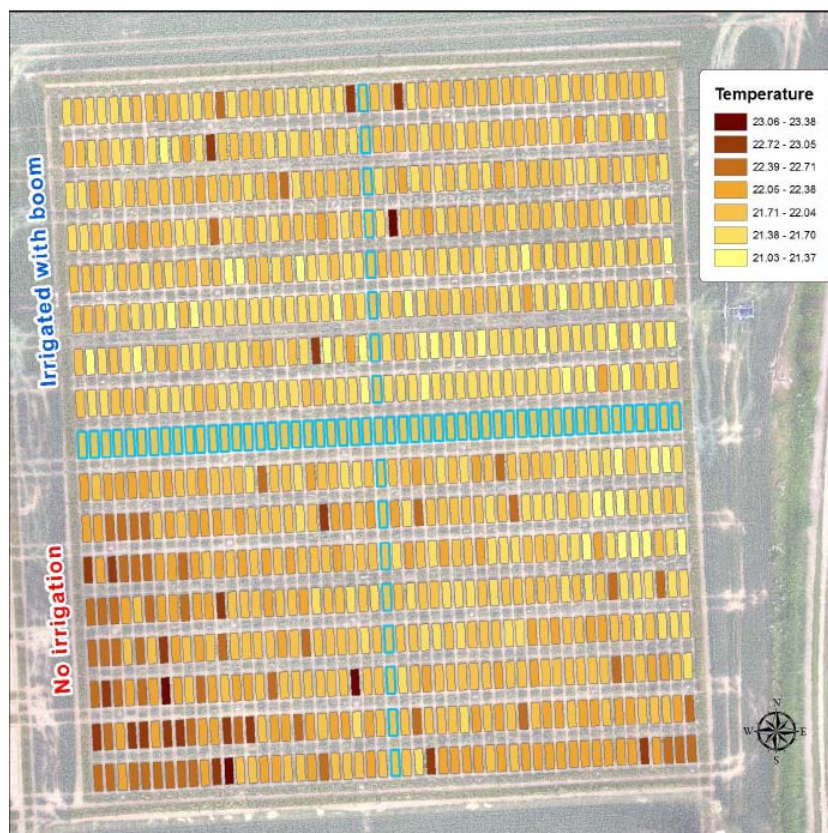
# 1. UK drought tolerance in Paragon x Garcia



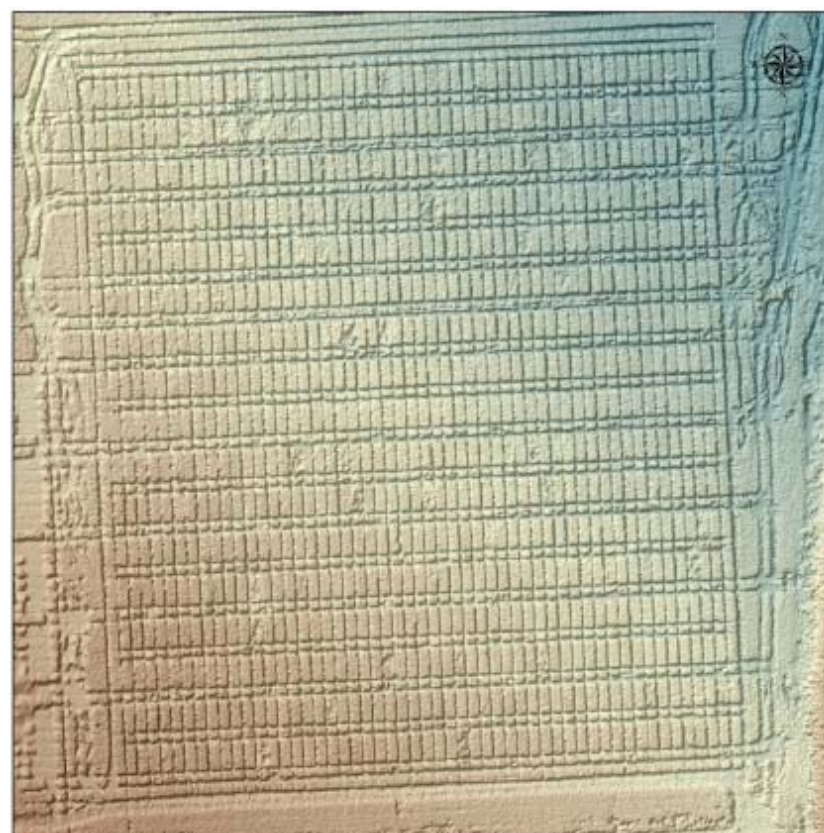
Many thanks to:  
Andrew Riche and March Castle for flying /data capture,  
Adam Michalski for image processing  
Malcolm Hawkesford for funding.

# 1. UK drought tolerance in Paragon x Garcia

Temperature



Height above sea



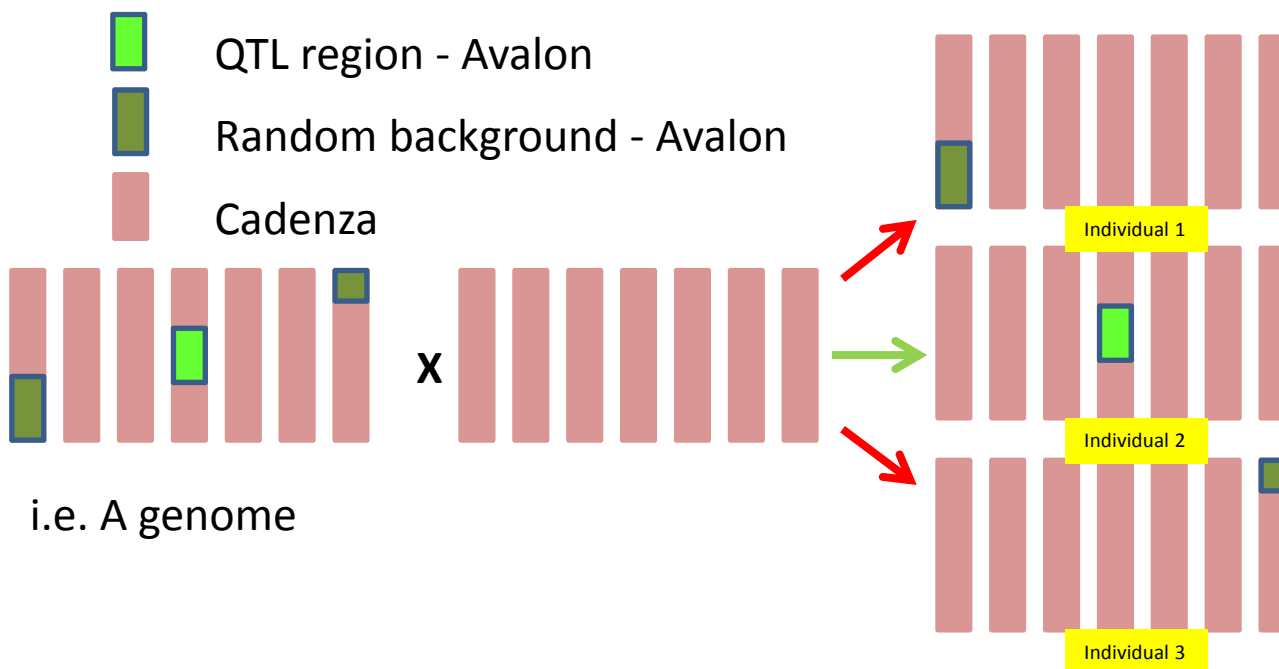
- No QTL identified with drone canopy temperature data.
- Images suggest differences in temp mainly due to topology of field?

# 1. UK drought tolerance in Paragon x Garcia

- Drought Trial to be repeated 2016-2017
- Riverside Field, Church Farm, Bawburgh - field with lighter soil and gentler slope
- Will monitor soil water content from autumn 2016
- Will use drone to monitor emergence from autumn 2016

## 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

- Plan to **'tile'** the whole genome with chromosome segments to make recombinant substitution lines for the whole genome, in both Avalon and Cadenza backgrounds.



## 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

Pilot experiment  
with 18 NILs

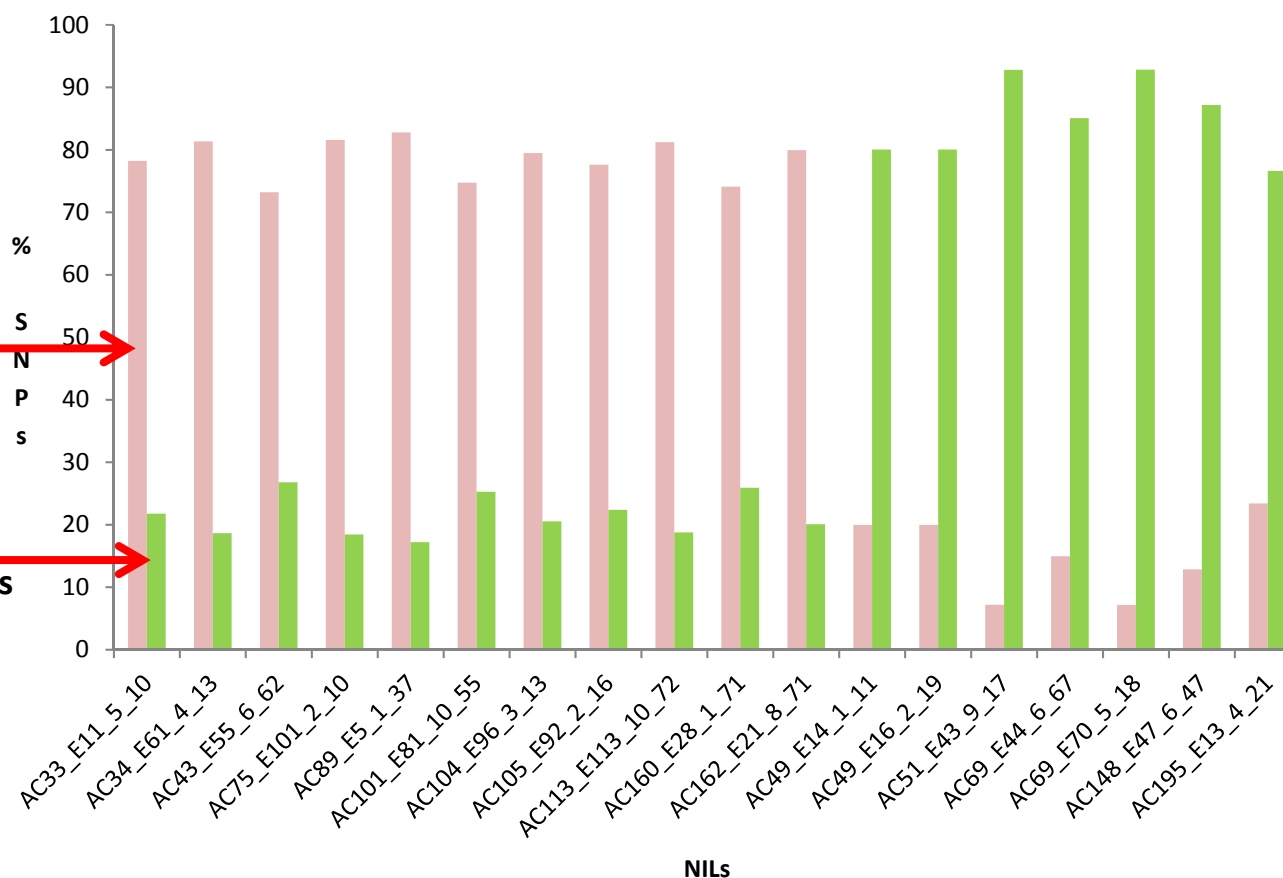
1D EM	AC33_E11_5_10_1A
3B YLD	AC34_E61_4_13_1A
6A Ht	AC43_E55_6_62_1A
6B EM/Ht	AC75_E101_2_10_1A
6A Ht	AC89_E5_1_37_1A
6B Ht	AC101_E81_10_55_1A
1B EM	AC104_E96_3_13_1A
7B YLD	AC105_E92_2_16_1A
3A Ht	AC113_E113_10_72_1A
3B Ht	AC160_E28_1_71_1A
2D Ht	AC162_E21_8_71_1A
5A YLD	AC49_E14_1_11_1A
1D EM	AC49_E16_2_19_1A
2D YLD	AC51_E43_9_17_1A
3A Ht	AC69_E44_6_67_1A
7D YLD	AC69_E70_5_18_1A
2D Ht	AC148_E47_6_47_1A
1B EM	AC195_E13_4_21_1A

## 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

- 18 NILs genotyped on 820K array to determine the *amount* and *location* of random background

i.e.  
~78% Cadenza background

~22% Avalon  
QTL region + random segments



# A chromosome segment substitution library (CSSL) for Avalon x Cadenza

- Generated genetic maps of all the NILs to determine the extent and location of the QTL regions and random segments
- Maps produced for the 18 NILs using the Framework map (1286 markers) generated by Winfield *et al* (2015)\*
- The maps consist of 1260 Markers for each NIL
- Maps are available on WGIN website <http://www.wgin.org.uk/>

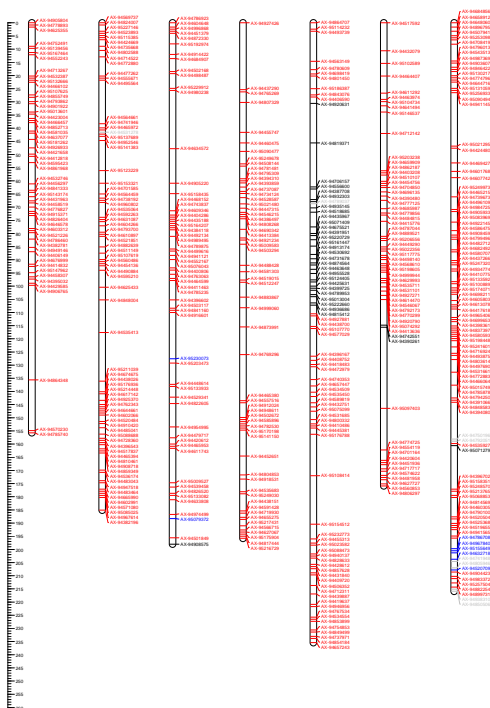
\*“High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool” Winfield *et al* 2015. **PBJ**



# 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

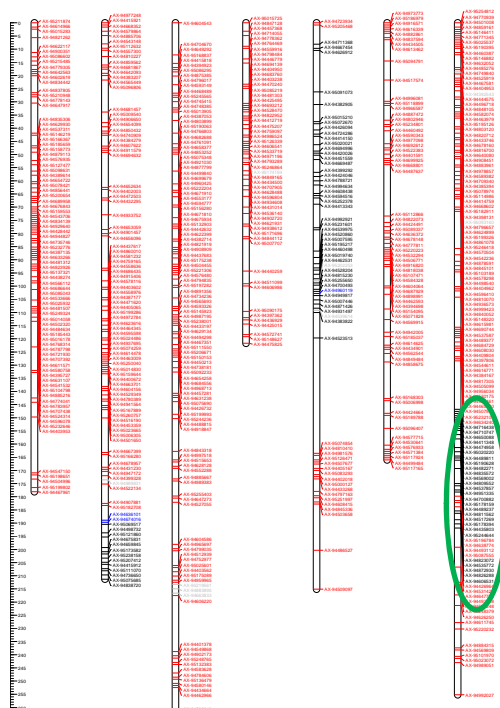
## A Genome

AC105\_E92.2\_16.7B YLD Cadenza background  
AC105\_E92.2\_16.1A AC105\_E92.2\_16.2A AC105\_E92.2\_16.3A AC105\_E92.2\_16.4A AC105\_E92.2\_16.5A AC105\_E92.2\_16.6A AC105\_E92.2\_16.7A



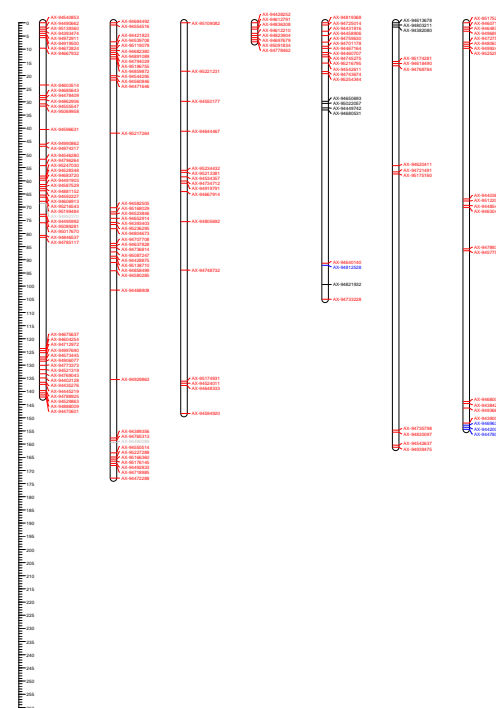
## B Genome

AC105\_E92.2\_16.7B YLD Cadenza background  
AC105\_E92.2\_16.1B AC105\_E92.2\_16.2B AC105\_E92.2\_16.3B AC105\_E92.2\_16.4B AC105\_E92.2\_16.5B AC105\_E92.2\_16.6B AC105\_E92.2\_16.7B



## D Genome

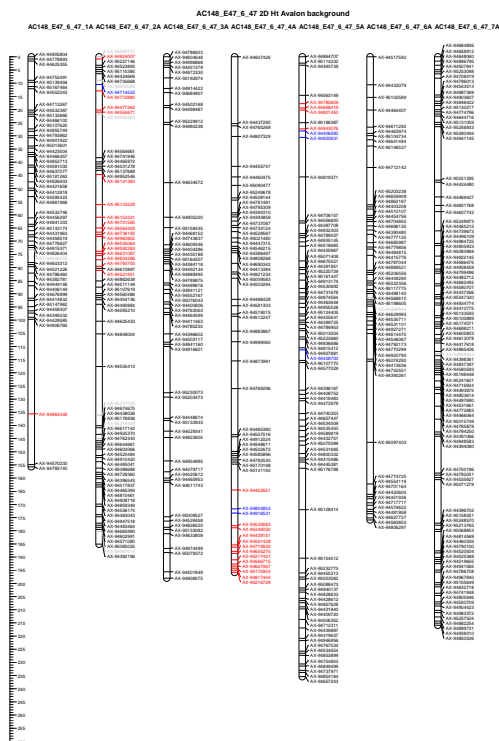
AC105\_E92.2\_16.7B YLD Cadenza background  
AC105\_E92.2\_16.1D AC105\_E92.2\_16.2D AC105\_E92.2\_16.3D AC105\_E92.2\_16.4D AC105\_E92.2\_16.5D AC105\_E92.2\_16.6D AC105\_E92.2\_16.7D



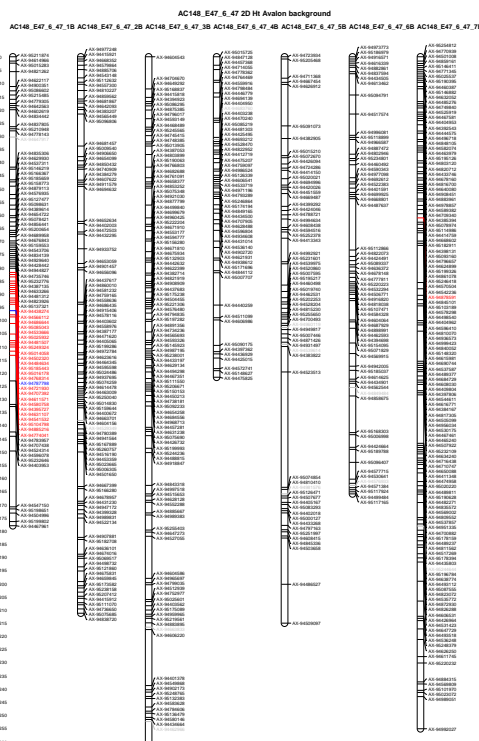
**Cadenza** background - **Avalon** random background and **QTL region**

# 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

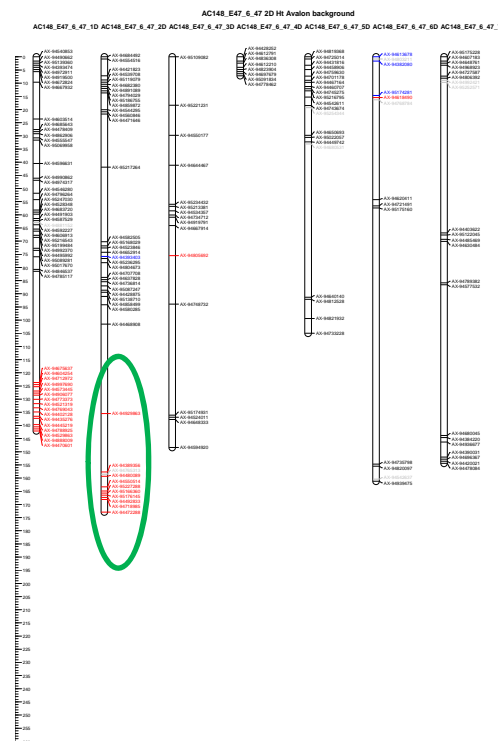
## A Genome



## B Genome

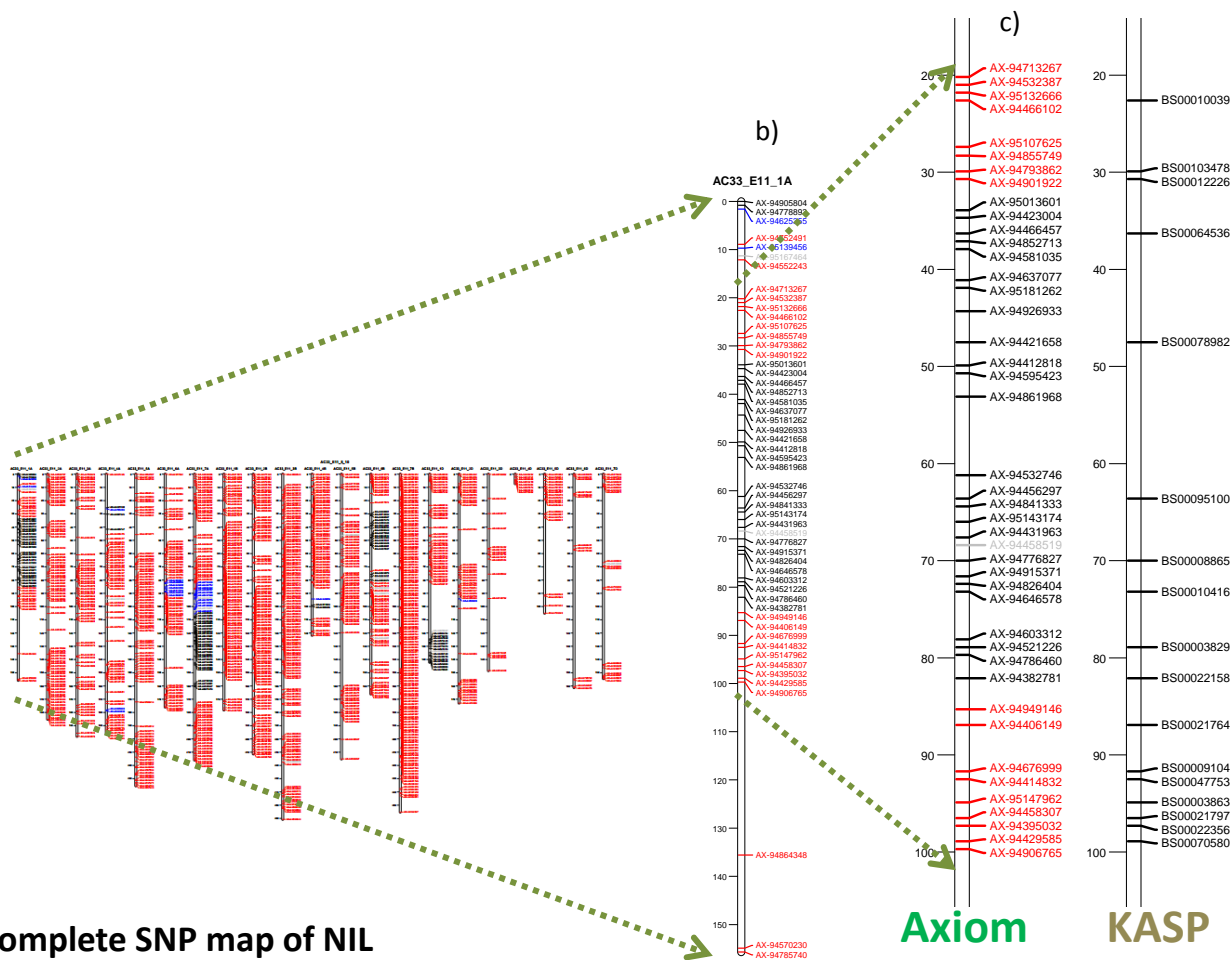


## D Genome



Avalon background - **Cadenza** random background and **QTL** region

# 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza



Complete SNP map of NIL  
With QTL for 1D DTEM

Chromosome 1A

Axiom KASP

Section of Chromosome 1A  
Showing the Axiom® SNP markers  
and the BS KASP markers from the  
University of Bristol Genomics Facility

## 4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

- Genotyping carried out on 94 NILs (+ Avalon and Cadenza) using Breeders 35K array
- Estimate of % coverage of background (+ foreground) for each chromosome
- Using AxC map positions of markers
- But may need to remap some as order not correct
- NILs backcrossed to the recurrent parent to generate lines where QTL region and random segments have been separated
- Will select specific lines to follow as some duplicated segments

	Avalon	Cadenza
	43 lines	46 lines
1A	~80%	~60%
1B	~95%	~90%
1D	~30%	~70%
2A	~80%	~95%
2B	~60%	~90%
2D	~95%	~70%
3A	~95%	~95%
3B	~70%	~95%
3D	~80%	~60%
4A	~80%	~75%
4B	~80%	~85%
4D	~80%	~95%
5A	~90%	~75%
5B	~80%	~90%
5D	~95%	~80%
6A	~95%	~85%
6B	~95%	~95%
6D	~95%	~95%
7A	~60%	~70%
7A or 7D	~40%	~90%
7B	~60%	~90%
7D	~95%	~30%
<b>INCLUDES FOREGROUND</b>		

# WGIN 3

## Update on stability and resilience

Malcolm J. Hawkesford

6<sup>th</sup> Management Meeting  
11<sup>th</sup> October 2016



# Nitrogen Use Efficiency (NUE), stability of wheat grain yield, wheat grain quality and canopy longevity

## (Work packages 2.3, 2.4, 3.4, 4.3, 4.4 Milestones 9 and 10)

---



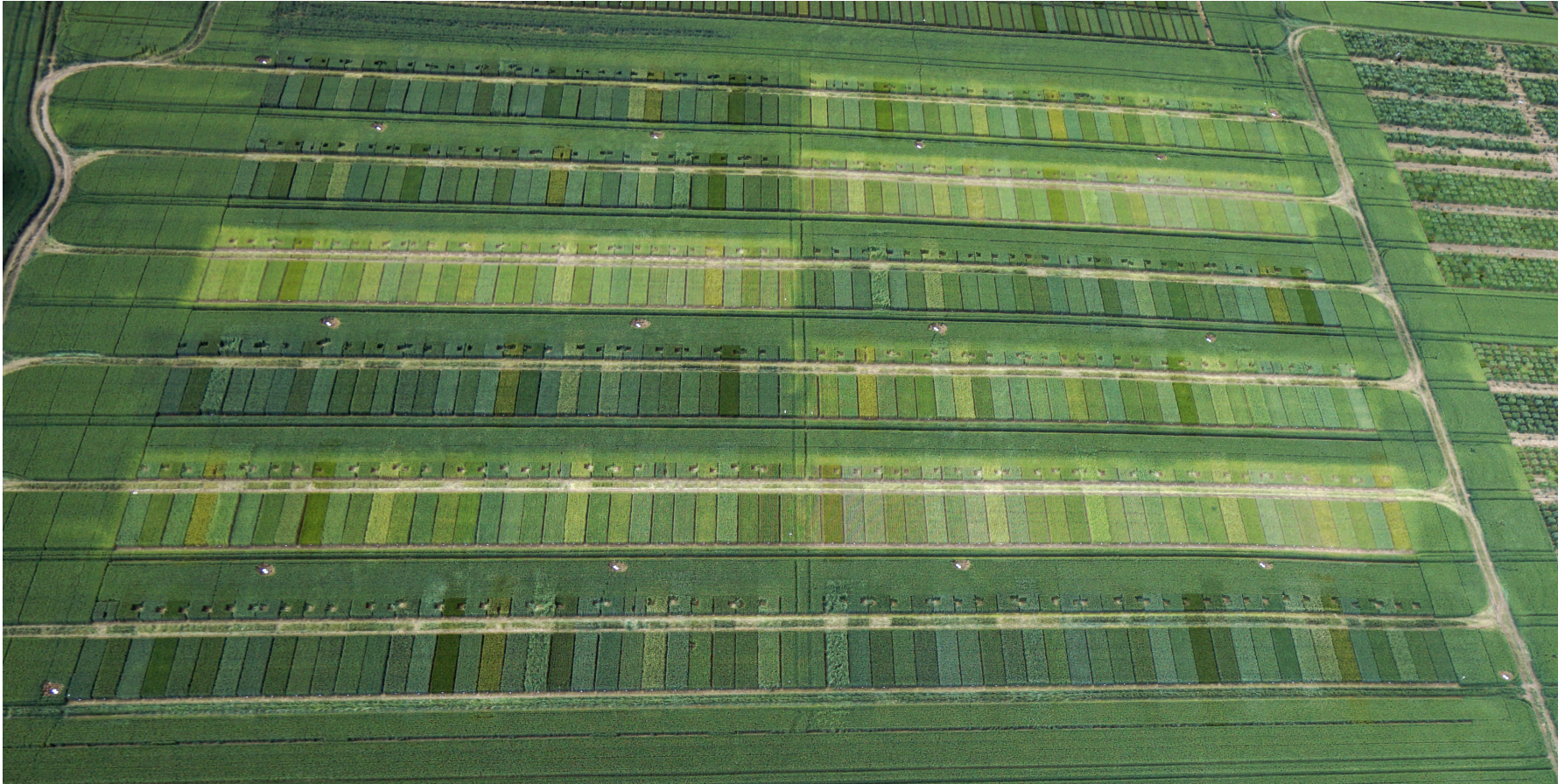
ROTHAMSTED  
RESEARCH

- The existing dataset includes yield, nitrogen use efficiency (NUE) and associated crop development data. In the next two years this data set will be extended, and the whole dataset analysed finally with respect to **stability and resilience** of these key agronomic traits, drawing on meteorological data from the Rothamsted site
- In 2015 and 2016, **post anthesis uptake of nitrogen and minerals** will be evaluated (mineral composition will be determined by ICP atomic absorption spectroscopic analysis to provide data on a wide range of minerals including P, S, K, Ca, Mg, Fe and Zn).
- **Archive WGIN material** from the previous 3 years (2012-14) (grain and straw) of final harvest grain and straw to give a 5 year dataset on mineral uptake and final partitioning.

# The Rothamsted WGIN Diversity experiment (since 2004; drilling 14<sup>th</sup> year)

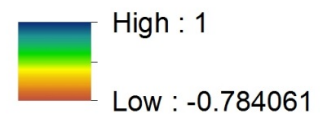
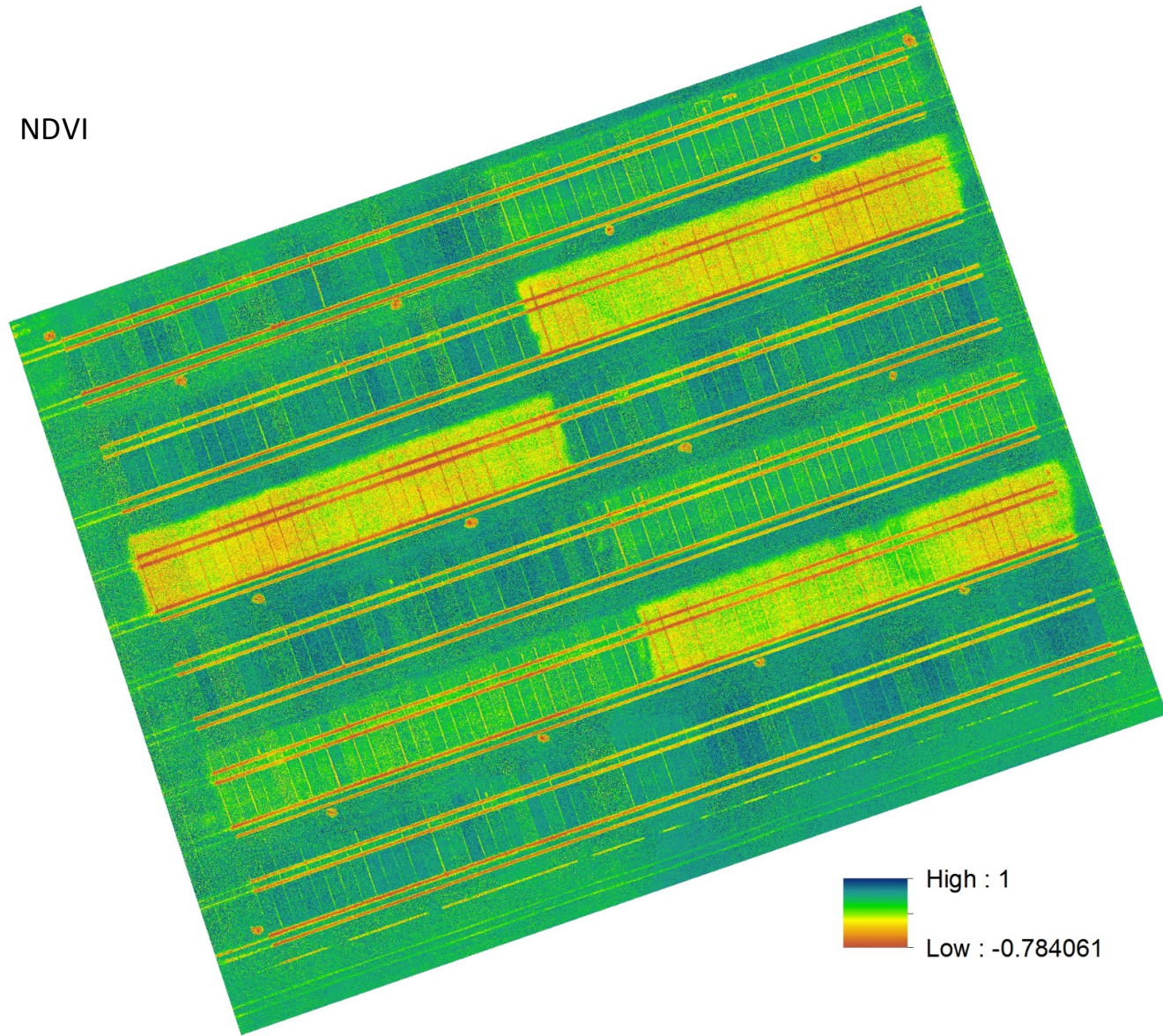


ROTHAMSTED  
RESEARCH



WGIN 3

NDVI



WGIN 3



Wheat varieties for WGI/20:20 Wheat/DFW NUE experiment 2016/17

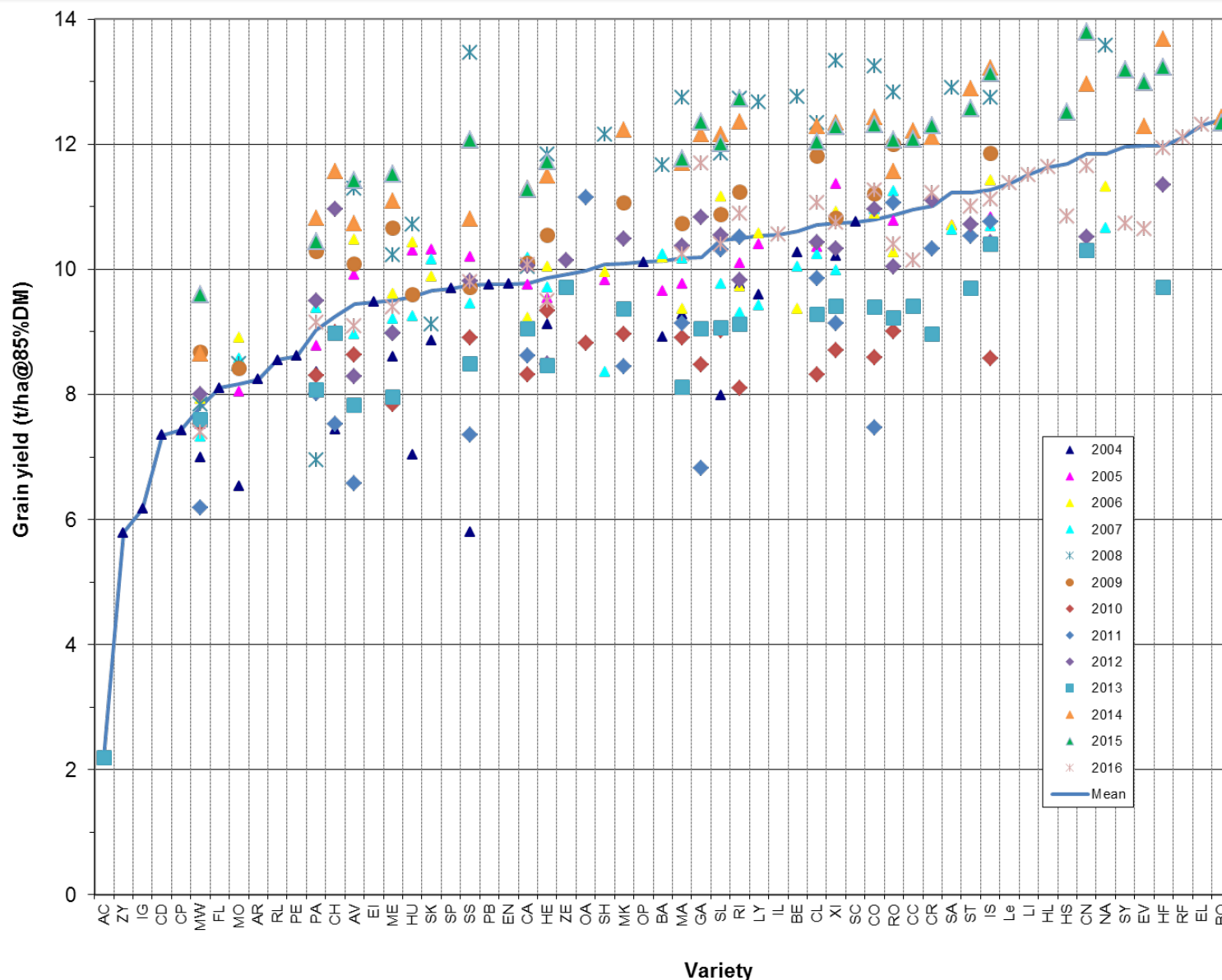
WGIN 3

14 <sup>th</sup> year					NB lost KWS Evoke and Stigg this year	
Variety	Source	Code	Nabim	Rationale	inclusion in trial requested by	Previous years of trials (harvest year)
1. Avalon		AV	1	WGIN DH parent; Low NupE & NutE (D) <b>WUE trial</b>	PB, RG, MJH	05-16
2. Cadenza		CA	2	WGIN DH parent; Best NupE (W) <b>WUE trial</b>	PB, RG, MJH	04-16
3. Claire	LIM	CL	3	Was biggest area on RL; WGIN DH parent; <b>Good second wheat</b>	PB,PS	05-16
4. Cocoon	Agrii/Secobra	CC	3	Tall variety. High yield. 2010 introduction. Eyespot and rust resistant.	MH	13-16
5. Conqueror	KWS	CN	4	New Grp 4, very high yielding	MH	12-16
6. Cordiale	KWS	CO	2	<b>Good second wheat. BBSRC Quality project WUE trial</b>	RG	06-16
7. Crusoe	LIM	CR	2	Carries diccooides. Shows the 'stay green' character		11-16
8. Evolution	Limagrain	EL	4	High yielding. Hard wheat. Consistent? Moderate straw length.	MH	16
9. Gallant	Syn	GA	1	new claimed high yield and high protein type	MH	10-16
10. Graham	Syn	GR	4	<b>High yielding</b>	<b>MH</b>	<b>New 2017</b>
11. Hereford	Syn	HF	4	Feed (not on RL), high yield, brown rust susceptible, possible low take-all build-up and good KHK/RG resistance. Multi trait.		12-16
12. Hereward	RAGT	HE	1	Best protein on RL; benchmark bread variety. <b>BBSRC Quality project WUE trial</b>	PB,PS	04-16
13. Hylux	Saaten Union	HL		Hybrid. Early flowering and maturing. Can be mildew susceptible; treat T0. Good under stress? Breadmaking?	MH	16
14. Hystar	Saaten Union		4	Hybrid for the first time, soft feed, high yield, good roots	PS	15-16
15. RGT Illustrious	RAGT	IL	can	Candidate for 2016/17. For breadmaking. Good quality and breadmaking ability even with low protein	MH	16
16. Istabraq	LIM	IS	4	Best yield on RL; Distilling cultivar; In LINK 'GREENgrain'; <b>Good second wheat. BBSRC Quality project. WUE trial</b>	PB,PS	05-16
17. Leeds	KWS	LE	4	soft	SG	16
18. KWS Lilli	KWS	LI	2	Very high yield.. Short and stiff straw.	MH	16
19. Malacca	KWS	MA	1	Biggest Group 1 area; DH choice; Low NupE, high NutE (W). <b>BBSRC Quality project</b>	PS	04-16
20. Maris Widgeon		MW	1	Tall (rht), old cultivar <b>WUE trial</b>	PB, AM	04-16
21. Mercia		ME	1	Low NupE & NutE (desk); Low Canopy N requirement; In IGF micro-array. <b>WUE trial. RHT series</b>	RG	04, 06-16
22. Paragon	RAGT	PA	1	Spring variety; WGIN mutagenesis population; High NupE (W)	PB	04-16
23. Reflection	Syngenta	RF	4	Early maturing. High yielding hard milling.	MH	16
24. Riband	RAGT	RI	3	WGIN DH parent; Distilling cultivar; In LINK 'GREENgrain'; High NutE (W)	RG	04-16
25. Robigus	KWS	RO	3	Best Group 3 yield; Best NUE, high NupE & NutE (D); <b>Good second wheat. WUE trial</b>	PB, AM	05-16
26. KWS Siskin	KWS	SI	2	<b>Consistent across regionx. In CINAG trial inc at NW</b>	<b>K Goulding</b>	<b>New 2017</b>
27. Skyfall	RAGT	SY	1	Still provisional RL as of June 2014 but very high yielding Grp 1	MH	15-16
28. Soissons	Elsoms	SS	2	WGIN DH parent; Early maturing; High NupE, low NutE (W) <b>WUE trial</b>	PB,RG, AM	04-16
29. Solstice	LIM	SL	2	Biggest Group 2 area; DH choice; Worst NupE (W)	RG	04-16
30. Xi19	LIM	XI	1	Best Group 1 yield; High NUE, NupE, NutE (D); Low NupE (W). <b>BBSRC Quality project. WUE trial</b>	PB,PS	04-16

# Variety yields (200 kgN/ha) 2004-16



ROTHAMSTED  
RESEARCH



WGIN 3

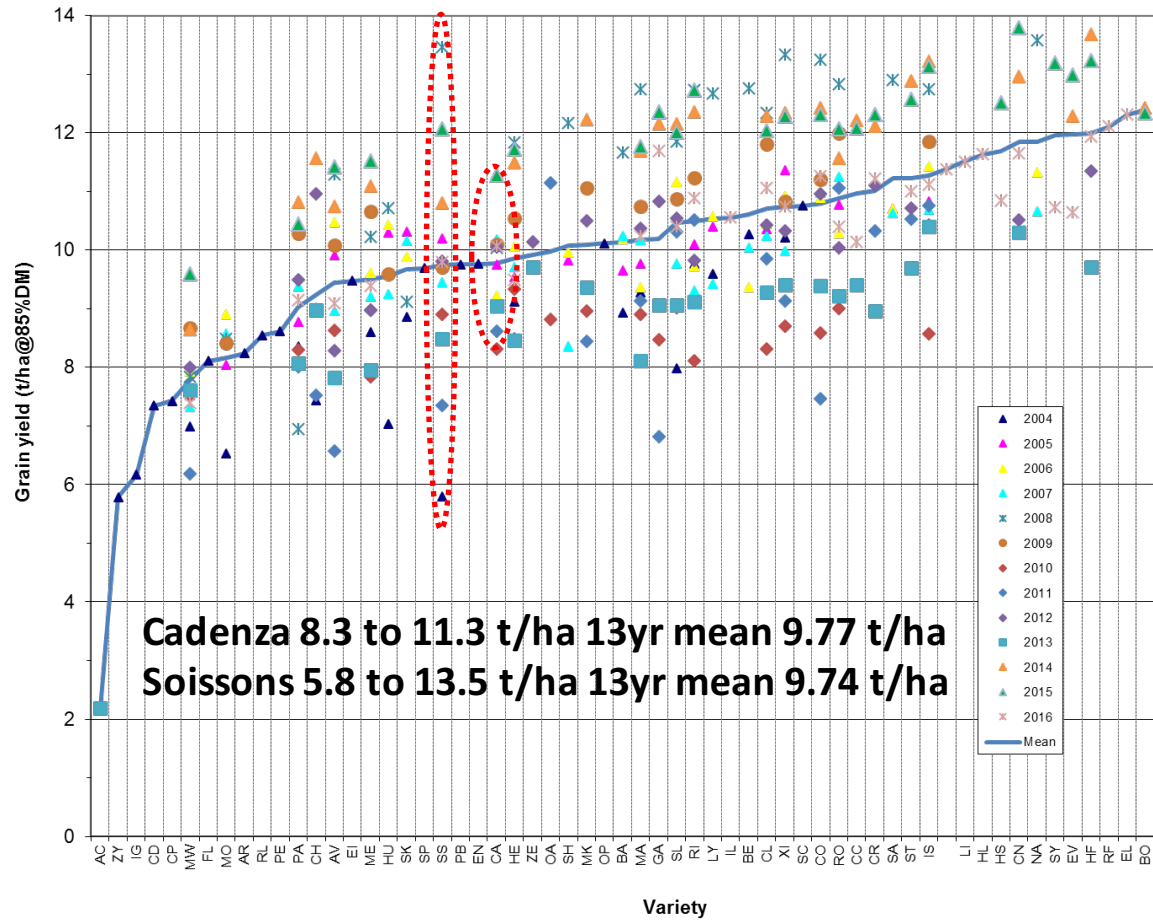


# WGIN Grain yields



ROTHAMSTED  
RESEARCH

Rothamsted WGIN N200 Grain yield 2004-2016

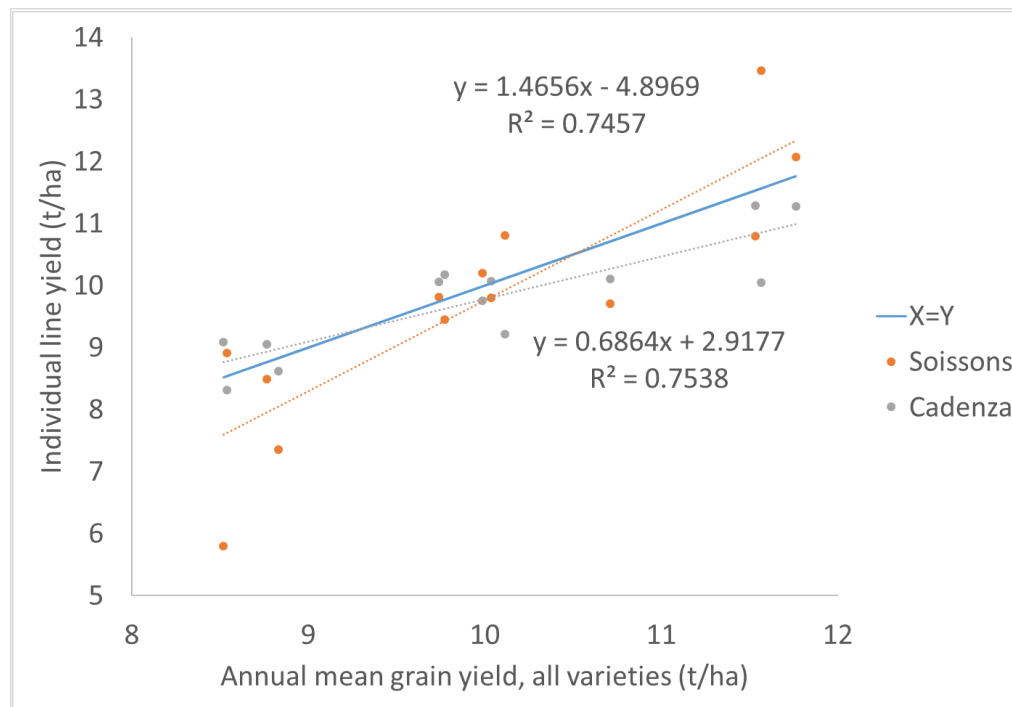


# Stress index – indicator of yield variability



ROTHAMSTED  
RESEARCH

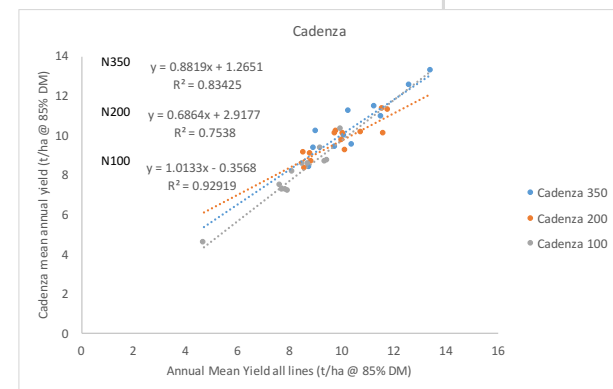
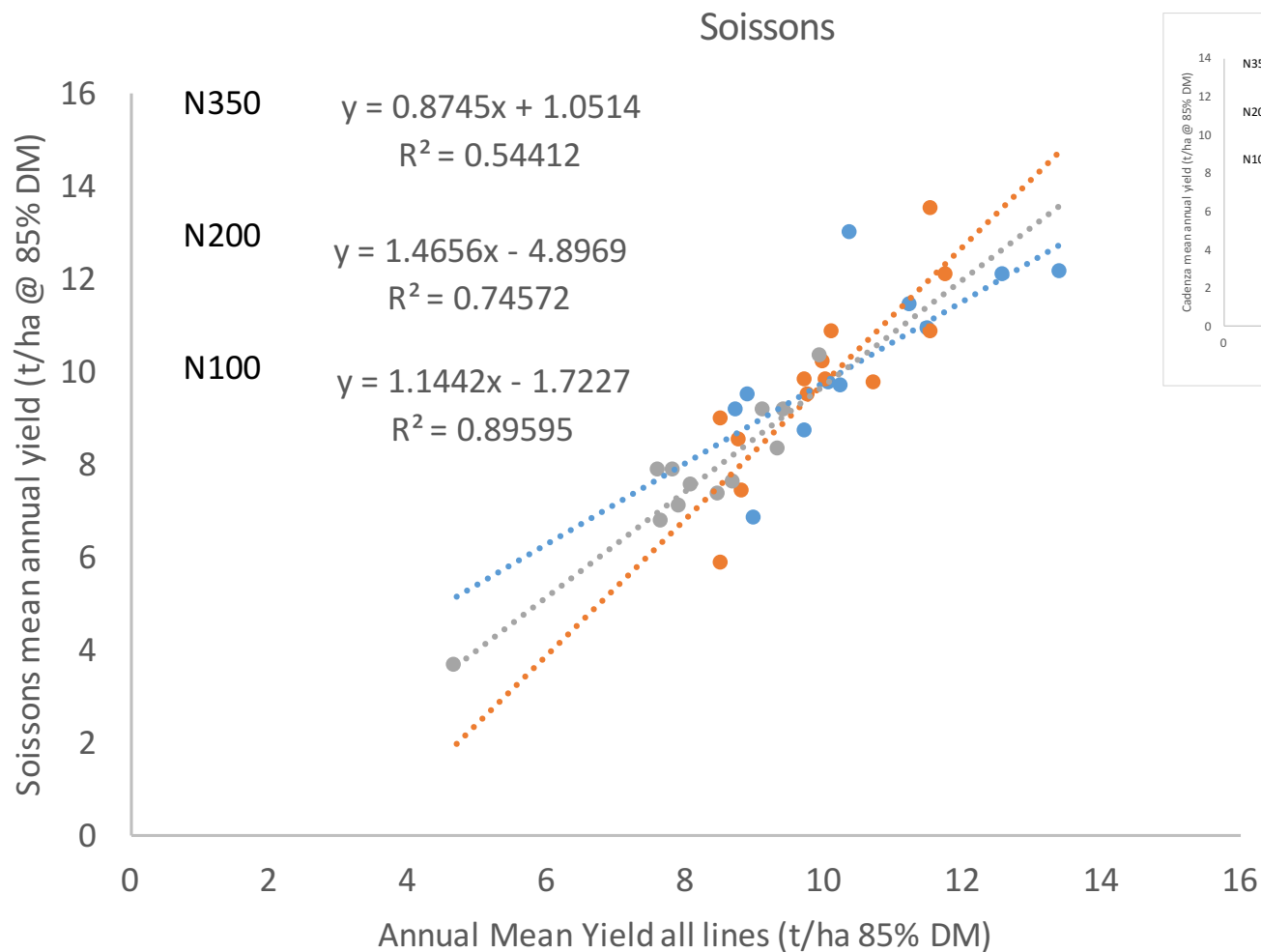
- Calderini and Slafer, 1999, Euphytica 107, 51-59.
- A variety with an annual yield equal to the annual mean of all varieties yield will have a slope of 1
- Cadenza shows reduced responsiveness to the environment, slope < 1
- Soissons shows increased responsiveness to the environment, slope > 1
- We can use the slope as an indicator of stress



# Stress index – nitrogen interaction



ROTHAMSTED  
RESEARCH



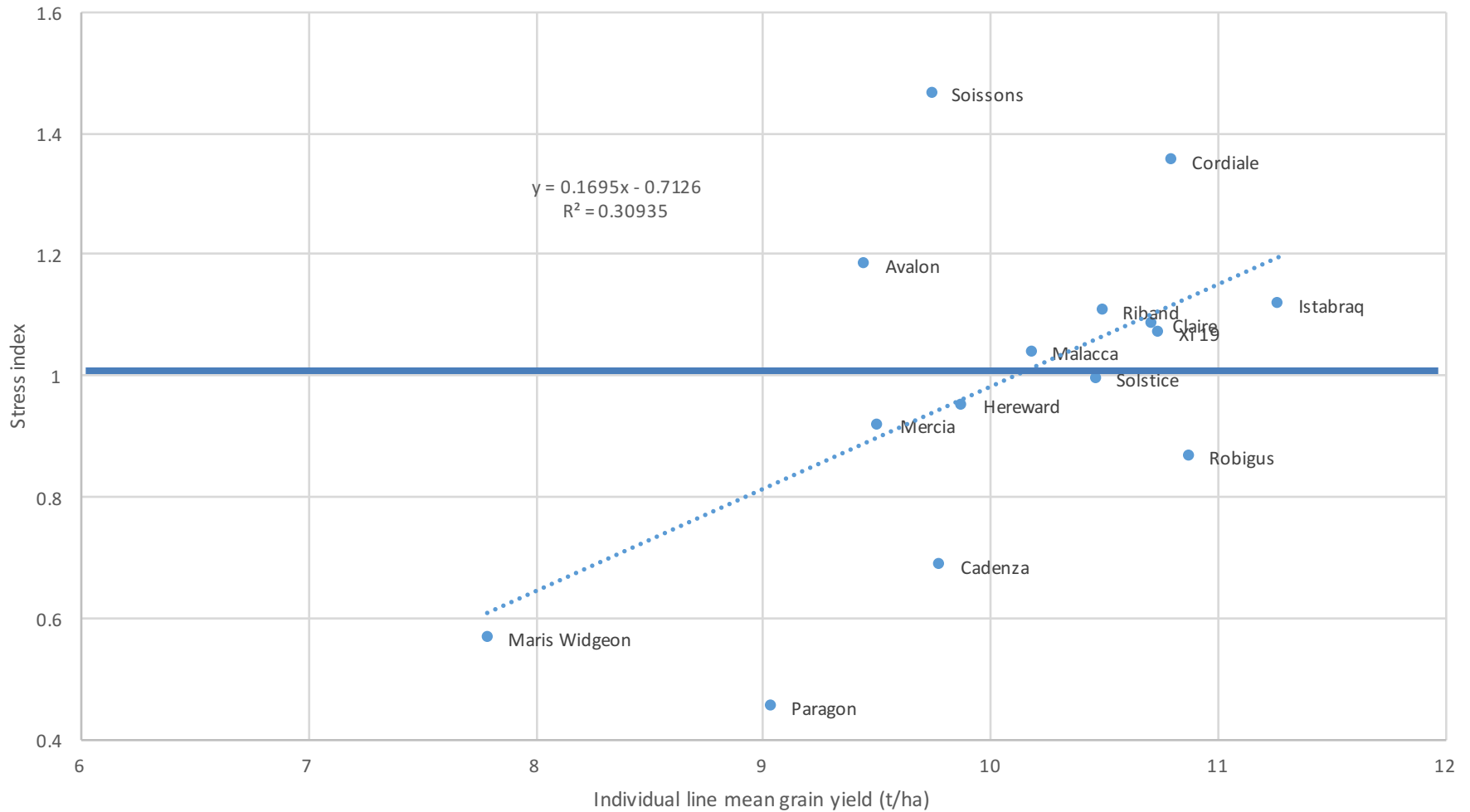
- Soissons 350
- Soissons 200
- Soissons 100

# Stress index



ROTHAMSTED  
CH

WGIN Diversity Stress index N200 - varieties grown for 11 or more yrs



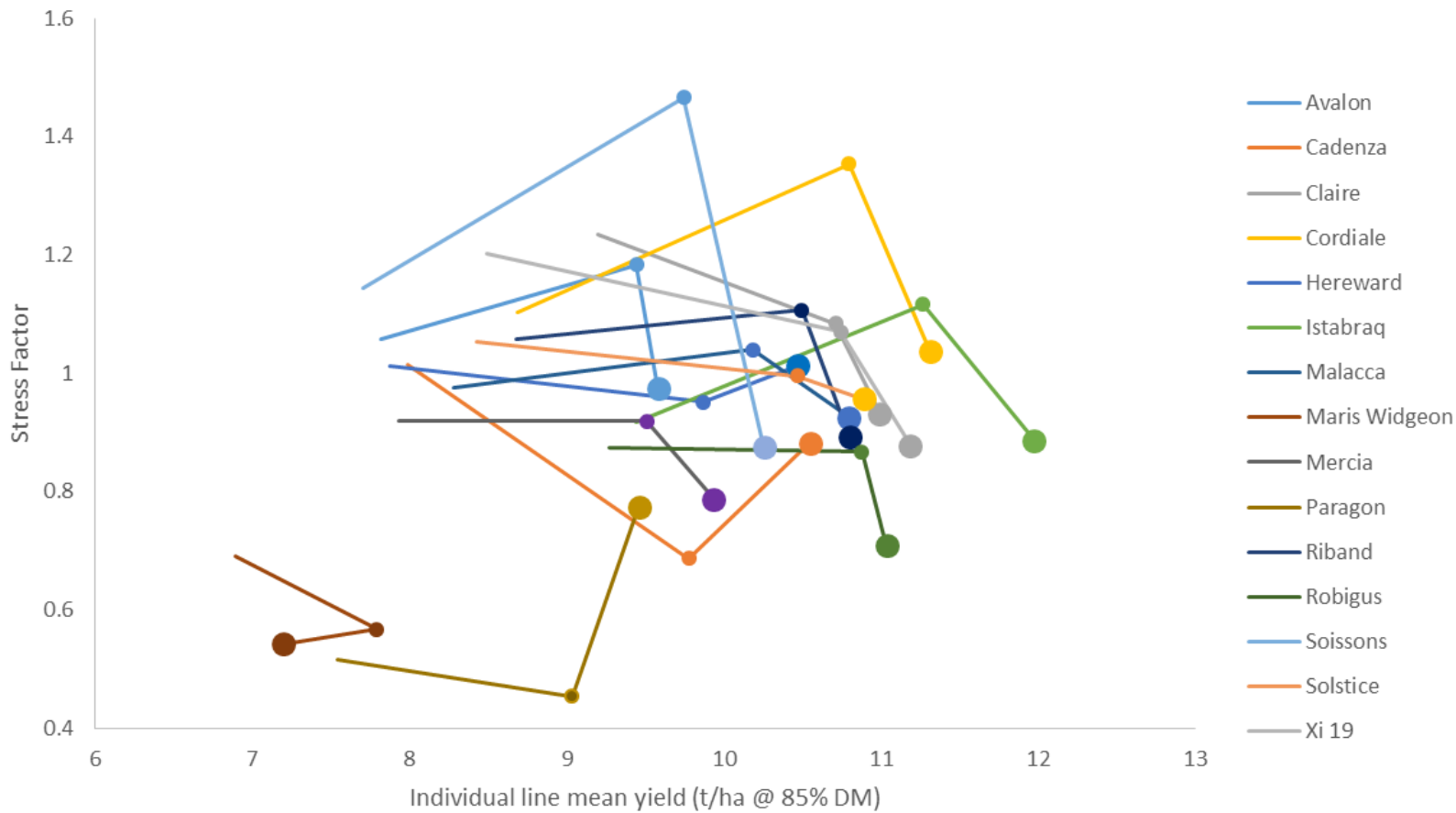
WGIN 3

# More N interactions



ROTHAMSTED  
RESEARCH

Stress Factors at 3 N levels, all LT Cvs (Marker size indicates N rate)



# WGIN3 Management Meeting

## 11<sup>th</sup> October 2016

### Screening germplasm for resilience to aphids (WP2.3)

Lesley Smart and Gia Aradottir





# Background to Aphid Work



ROTHAMSTED  
RESEARCH

Part of a BBSRC funded initiative to identify useful genetic variation from novel sources of wheat germplasm that can be used to improve modern wheat varieties. Wheat Improvement Strategic Programme (WISP) and also WGIN.

Screening wheat lines for evidence of resistance to the main cereal aphid pests in the UK – the bird cherry-oat aphid *Rhopalosiphum padi* and the grain aphid *Sitobion avenae*.

- Hexaploids from the Gediflux and Watkins collections (mostly from JIC)
- Tetraploid wheat lines (from NIAB)
- Synthetic wheat lines (from NIAB)
- Diploid wheat species including lines of
  - *Aegilops speltoides* (from JIC)
  - *Ae. tauschii* (from NIAB)
  - *Triticum monococcum* (from RRes WGIN).

  
Department  
for Environment  
Food & Rural Affairs

  
Wheat  
Genetic  
Improvement  
Network

  
WISP



# The Target Pests



ROTHAMSTED  
RESEARCH



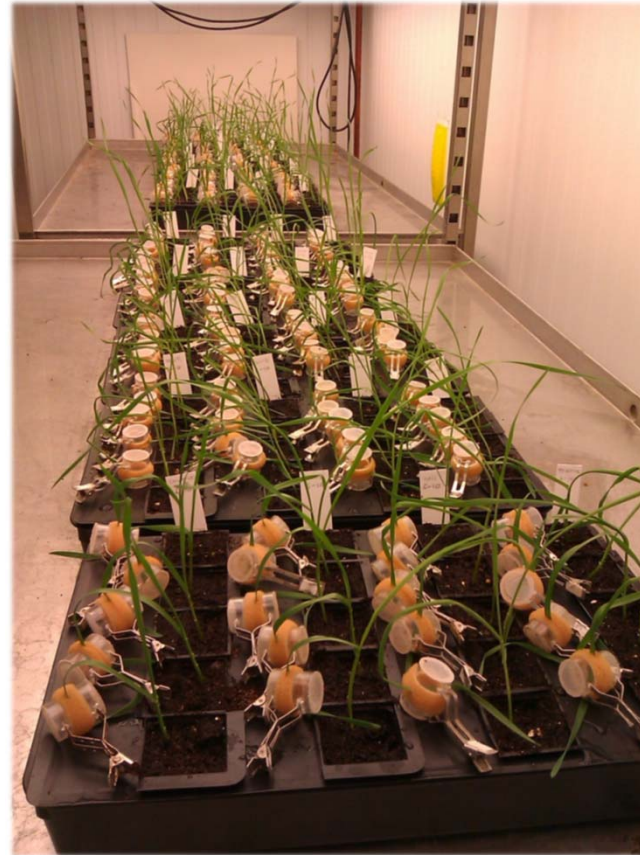
*Rhopalosiphum padi*



*Sitobion avenae*



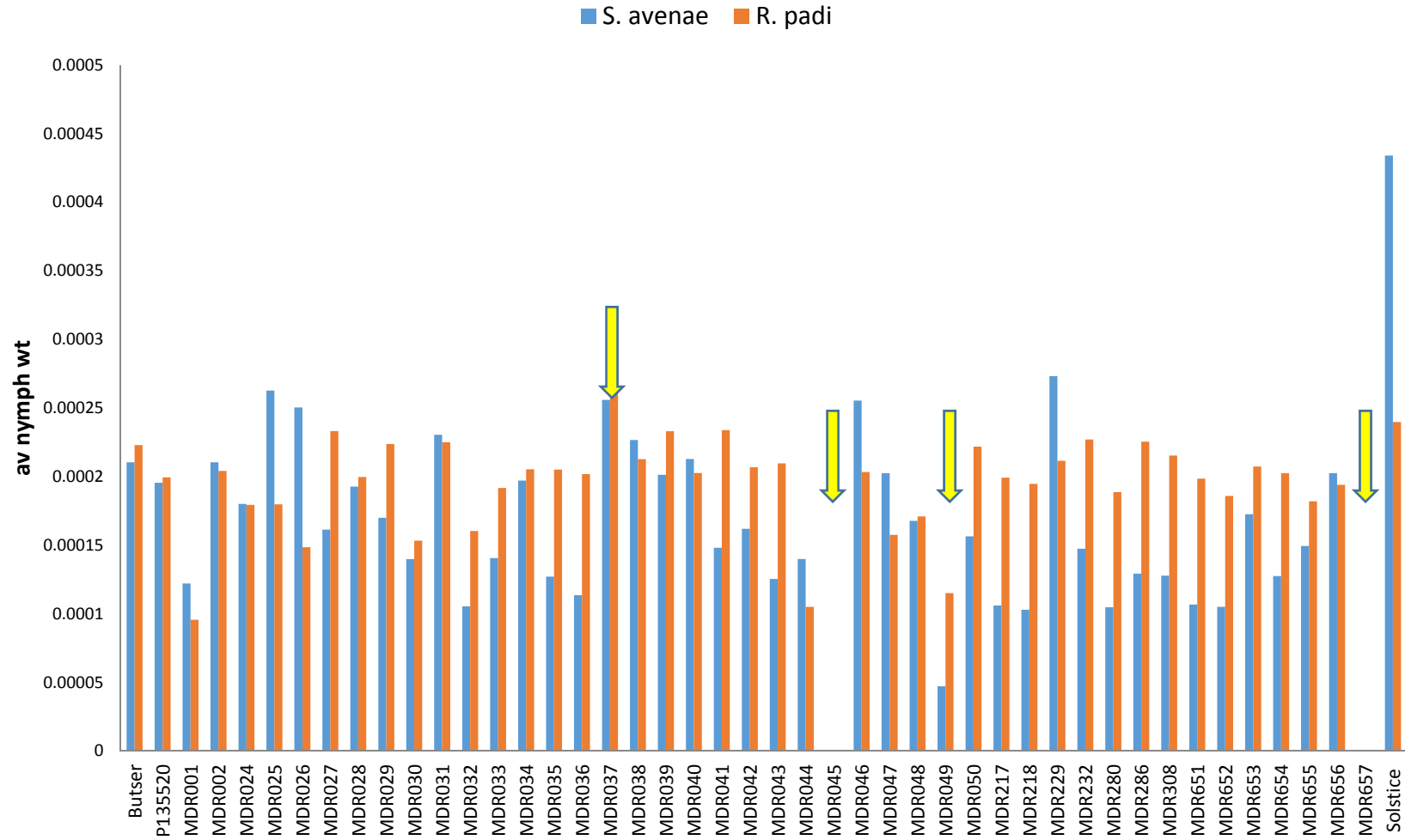
# Phenotyping Screen



Measure of aphid settlement, nymph production and nymph weight gain on a wide range of wheat germplasm

# Phenotyping Screen

## Nymph weight (g) on *Triticum monococcum* lines

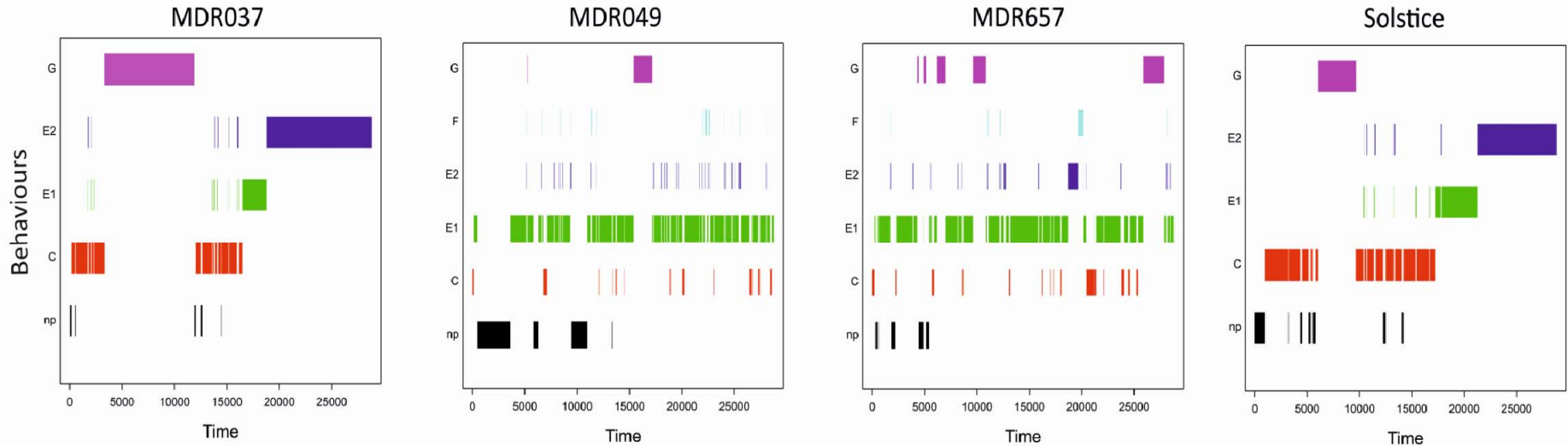
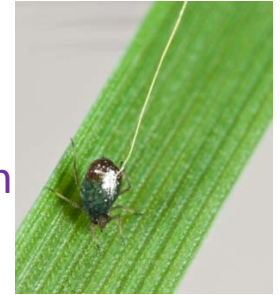


*T. monococcum* lines provided good evidence of resistance against both aphids



# Reduced feeding by *R. padi* in EPG bioassays in WISP

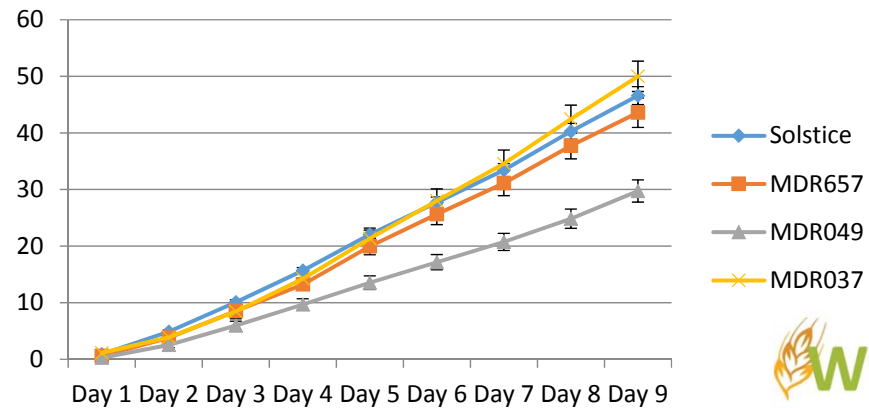
Behaviours: np: not probing, C: pathway phase, E1: salivation, E2: phloem ingestion (feeding), F: derailed stylet mechanics, G: xylem ingestion (drinking)



## Reduced fecundity of *R. padi*

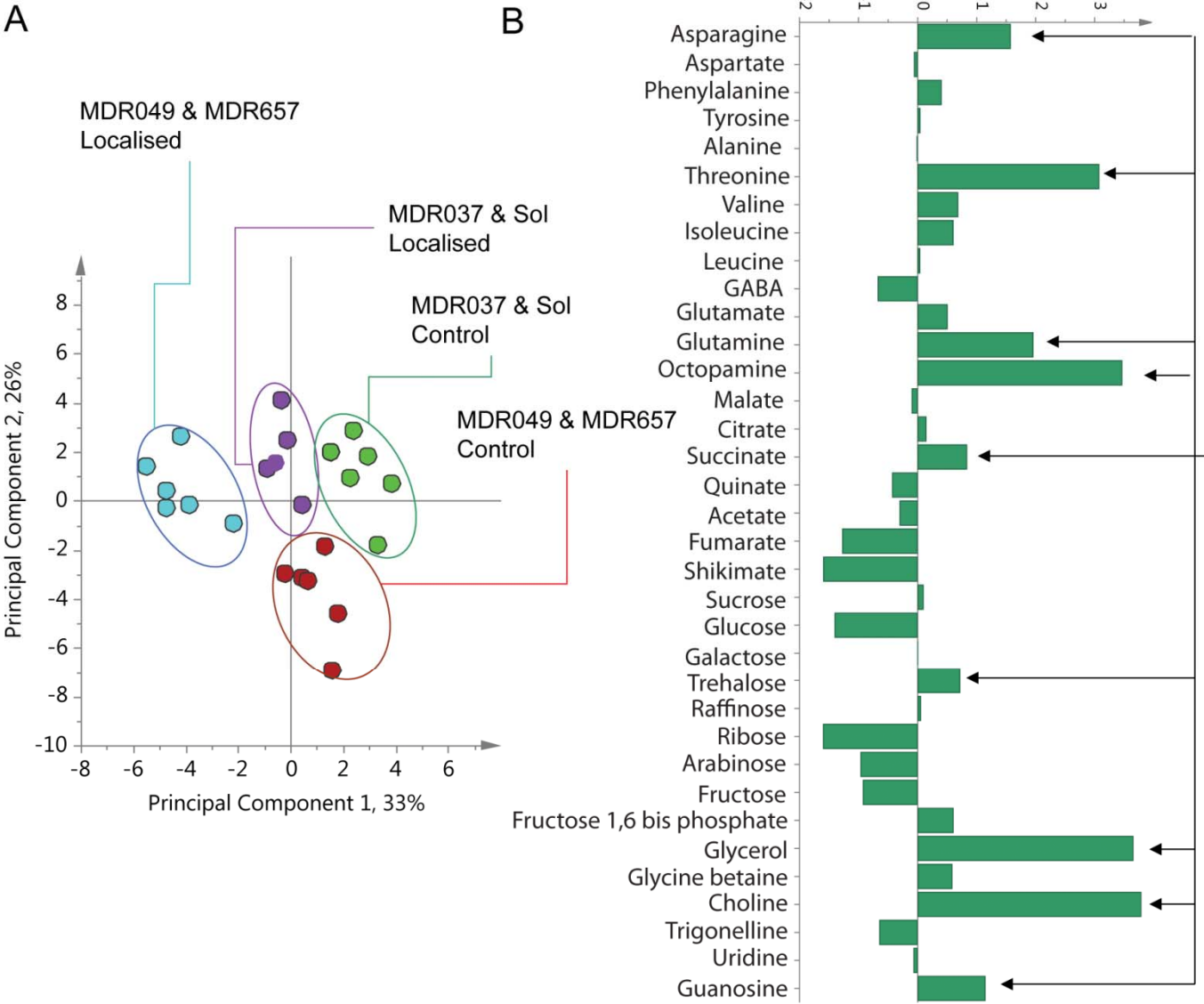
no nymphs produced on MDR045

### Cumulative nymph production



Greenslade *et al.*, (2016) *AAB*, 168, 435-449

# Metabolomic analysis of plant chemistry



Greenslade *et al.*, (2016) AAB, 168, 435-449

# Screening germplasm for resilience to aphids (WP2.3)

## Information to establish the likely genetic basis of resistance to cereal aphids

---



ROTHAMSTED  
RESEARCH

- Focus on *Triticum monococcum* lines as these provided the most promising leads for partial resistance to cereal aphids from work in WISP
  - Fewer nymphs were produced compared to hexaploid varieties
  - Reduced weight gain by the nymphs over time – disrupted feeding patterns
  - Slower development and population increase
  - Differential changes in plant chemistry in response to aphid damage

# Screening germplasm for resilience to aphids (WP2.3)

## Information to establish the likely genetic basis of resistance to cereal aphids

---



ROTHAMSTED  
RESEARCH

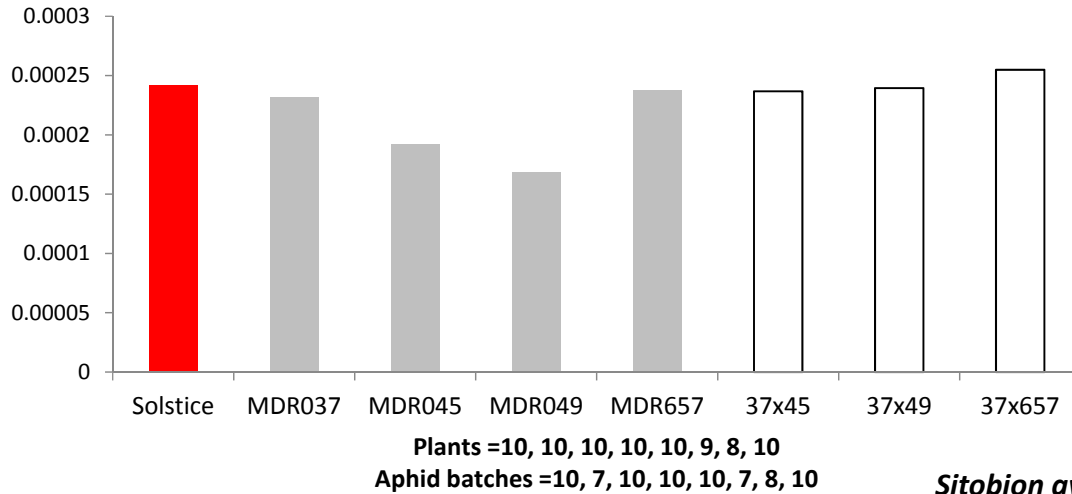
- Crosses of *T. monococcum* lines made by Mike Hammond-Kosack: MDR037 (susceptible) x MDR045, MDR049 and MDR657 (all showing evidence of partial resistance)
- F1 generations of these crosses tested in the phenotyping screen along with parental lines against both aphid species.
- F2 generations have now also been tested in the screen against both aphid species.



# Screening germplasm for resilience to aphids (WP2.3)

## Response to F1 generation of *T. monococcum* crosses

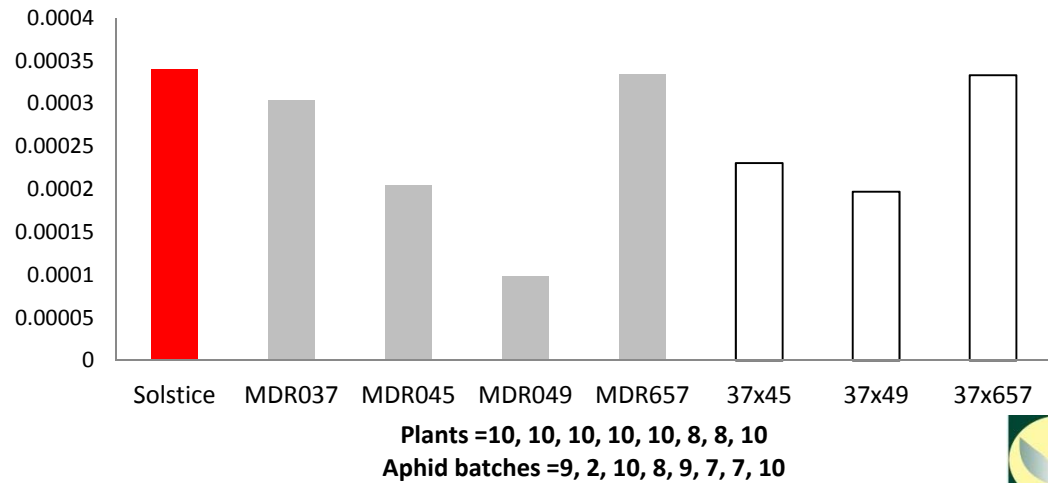
*Rhopalosiphum padi* mean nymph weight (g) after 6 days on *T. monococcum* parental lines and F<sub>1</sub> crosses



MDR037 (susceptible) x  
MDR045, MDR049 and  
MDR657 (all showing partial  
resistance)

Aphid response on some parental lines differed from original findings. MDR049 consistent reduction in nymph weight gain

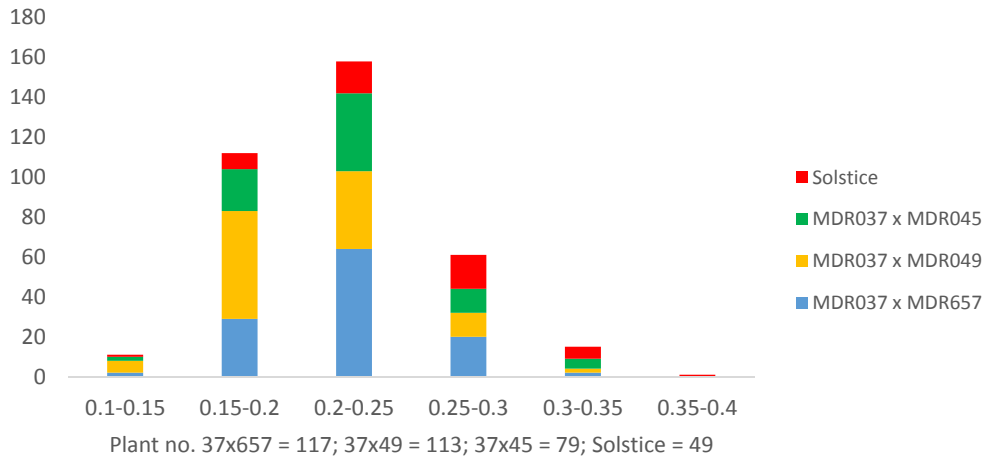
*Sitobion avenae* mean nymph weight (g) after 7 days on *T. monococcum* parental lines and F<sub>1</sub> crosses



# Screening germplasm for resilience to aphids (WP2.3)

## Response to F2 generation of *T. monococcum* crosses

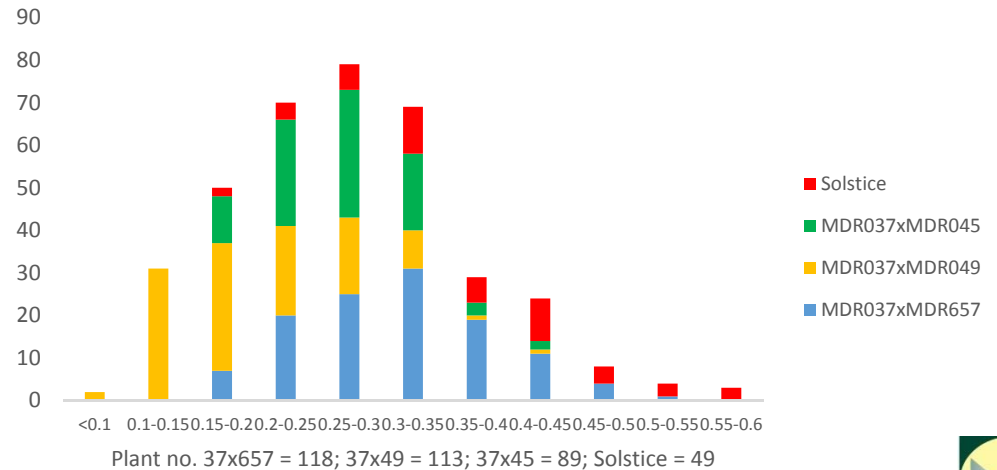
*Rhopalosiphum padi* on *T. monococcum* crosses F2s  
Number nymphs/weight range after 6 days (mg)



MDR037 (susceptible) x  
MDR045, MDR049 and  
MDR657 (all showing partial  
resistance)

Partial resistance (reduced weight gain) showing in F2 generation of MDR037 x MDR049, particularly for *S. avenae* the grain aphid.

*Sitobion avenae* on *T. monococcum* crosses F2s  
Number nymphs/weight range at 7 days (mg)



# Screening germplasm for resilience to aphids (WP2.3)

## Generation of F3 generation of *T. monococcum* crosses



# Summary WGIN3 (WP2.3)



ROTHAMSTED  
RESEARCH

- F1 generations of *T. monococcum* crosses tested in the phenotyping screen along with parental lines against both aphid species. Differences observed between responses of aphid species to F1s, but data limited. Aphid response on some parental lines differed from original findings. MDR049 consistent reduction in nymph weight gain.
- F2 generations of the crosses have also been tested in the screen. Some MDR037 x MDR049 F2s showing reduction in nymph weight gain, particularly for *S. avenae*. Little evidence of effects on nymph production.
- Backcrosses of F1s to MDR037 parent still to be tested.
- F3 generations have been harvested and will be screened in future and taken to F4 and beyond.

# Acknowledgements

Mike Hammond-Kosack  
Alex Greenslade  
Janet Martin



# WGIN 3

## Resistance to take-all and foliar diseases

Vanessa McMillan  
Kim Hammond-Kosack



Department  
for Environment  
Food & Rural Affairs

# Take-all disease



ROTHAMSTED  
RESEARCH

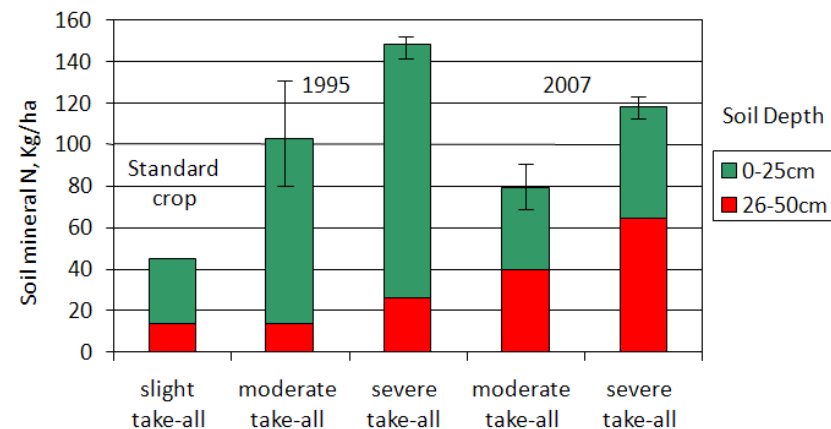
## Take-all disease – soil-borne fungus

*Gaeumannomyces graminis* var. *tritici* (Ggt)

## A major problem for 2<sup>nd</sup> / 3<sup>rd</sup> wheat crops



- Typical take-all patch showing stunting and premature ripening of the wheat crop
- Can decrease yield by up to 60%



- Decreases N uptake by the plant (Macdonald & Gutteridge, Plant and Soil, 2012)
- Environmental risk of nitrate leaching

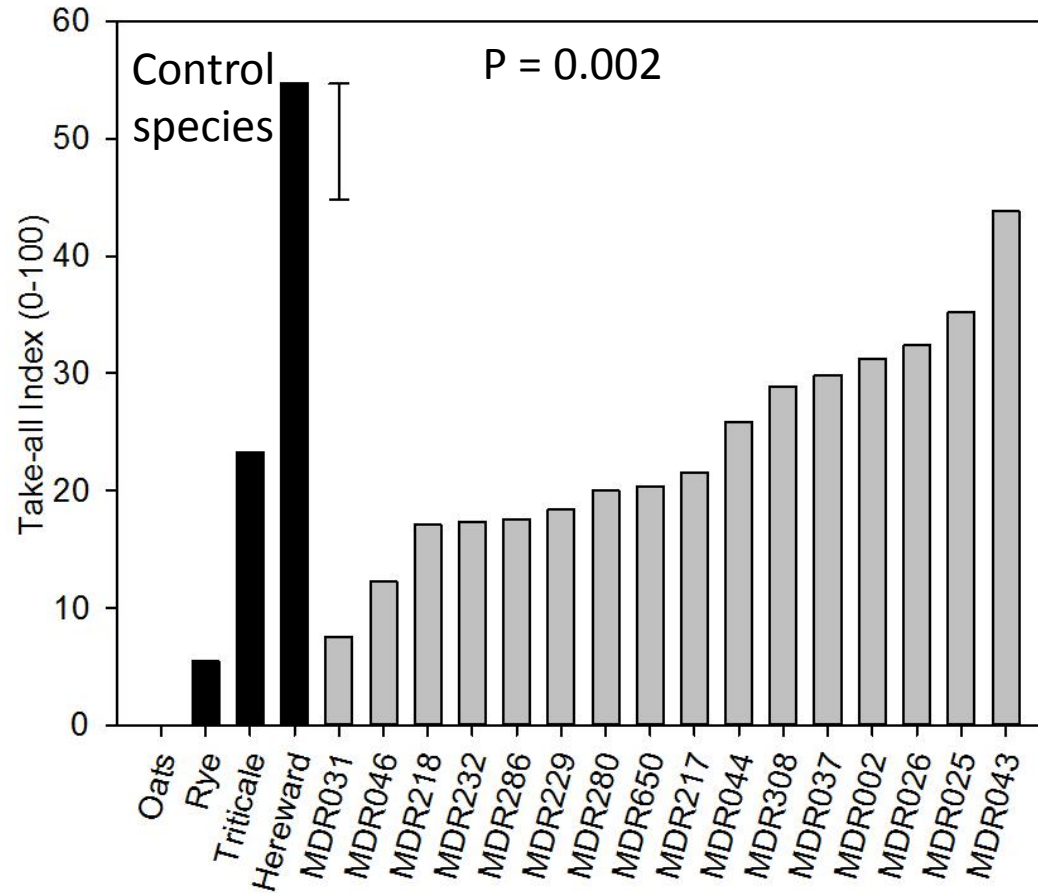


# Can root resistance to take-all be improved by using an ancient wheat?



ROTHAMSTED  
RESEARCH

- 3<sup>rd</sup> wheat field trials 2006-2011 (WGIN 1 and 2)
- **34 *T. monococcum* accessions (AA diploid genome)** tested over 5 years





# Development of *Triticum monococcum* mapping populations



ROTHAMSTED  
RESEARCH

## F<sub>2</sub> Tm cross progeny numbers:

Parentage		Estimated F <sub>3</sub> progeny number
<b>MDR031 (R) x MDR043 (vS)</b>	31 ears from 3 plants	450 ←
MDR031 x MDR229	16 ears from 1 plant	320
MDR031 x MDR650	48 ears from 3 plants	900
MDR043 (vS) x MDR031 (R)	48 ears from 3 plants	960
<b>MDR043 (vS) x MDR046 (R)</b>	36 ears from 3 plants	750 ←
MDR229 x MDR031	94 ears from 6 plants	2000

Populations advanced by SSD from F<sub>4</sub> to F<sub>6</sub> for future field phenotyping ←

# *Triticum monococcum* MDR031(R) x MDR043 (S) mapping population



ROTHAMSTED  
RESEARCH

- 516 F<sub>2</sub> seeds → 380 F<sub>6</sub> lines
- 0.5 – 33.5 g of each line available
- **129 lines** > 18 g seed selected for field trial + parents + Hereward control plots
- Plot size 4 rows x 0.6 m length, 80 seeds per plot
- Randomised block design (3-5 reps/genotype)
- **3<sup>rd</sup> wheat field trial** drilled in Claycroft 6<sup>th</sup> Oct 2016



# Resistance to multiple foliar diseases



ROTHAMSTED  
RESEARCH

## Watkins 2008 Field Trial

**10 Watkins accessions** with a high degree of resistance to all 4 foliar pathogens

Also a high take-all disease year with root infection early in the season

Was the foliar disease resistance an induced plant response?

Accession	Growth habit	Country of Origin	2008 Disease assessments			
			Yellow rust	Brown rust	Septoria	Mildew
18	Spring	India	0	0	T	T
137	Spring	Australia	T	T	0	T
203	Winter	India	0	0	0	T
231	Spring	Hungary	0	0	T	0
262	Spring	Canary Islands	0	0	0	0
399	Spring	China	T	0	T	0
495	Spring	Morocco	0	0	T	0
610	Spring	Yugoslavia	0	0	T	T
733	Spring	Iran	T	T	T	T
786	Spring	USSR	0	T	T	0

0 – no disease , T = trace



# WGIN 3 Watkins foliar disease experiments



ROTHAMSTED  
RESEARCH

- 10 Watkins lines + controls sown in both **1<sup>st</sup> wheat (no take-all)** and **3<sup>rd</sup> wheat (high take-all)** field trials in autumn 2014 and 2015
- No fungicides applied to allow natural disease to develop
- Score for foliar diseases + take-all

# Watkins foliar disease field trial 2015



ROTHAMSTED  
RESEARCH



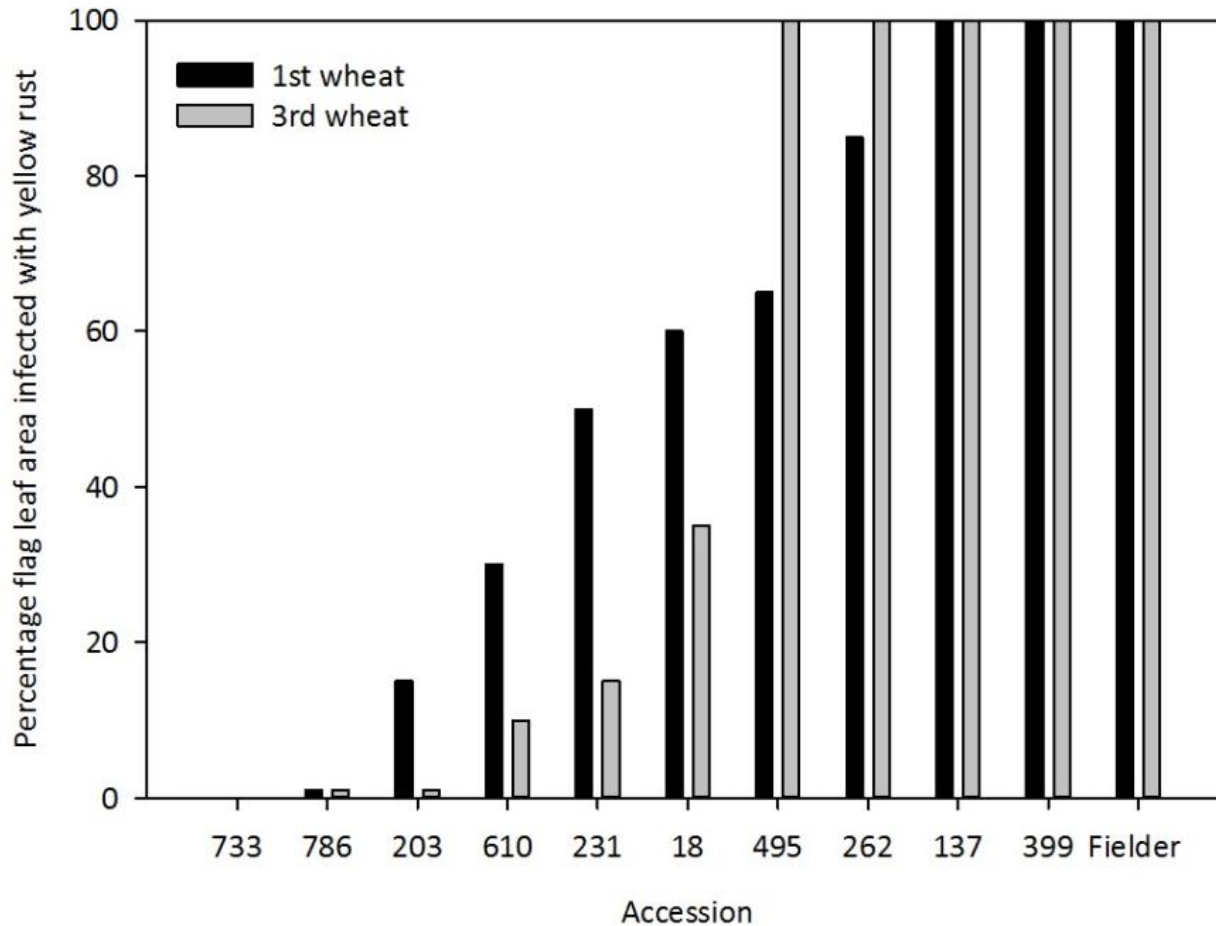
26<sup>th</sup> June 2015      1<sup>st</sup> wheat      Long Hoos 4

Yellow rust dominant disease that developed across 2015 field trials

# Evidence of resistance to yellow rust



ROTHAMSTED  
RESEARCH



- Evidence of induced response due to take-all?

# Evidence of resistance to yellow rust



ROTHAMSTED  
RESEARCH



Watkins 203

Low levels of yellow rust sporulation



Watkins 733

No sporulation

# Watkins foliar disease trial 2016

---



ROTHAMSTED  
RESEARCH

- Repeat field trial with 5 replicates of each genotype sown in autumn 2015 in Long Hoos 5 (1<sup>st</sup> wheat) and Long Hoos 6/7 (3<sup>rd</sup> wheat)
- Yellow rust, septoria and brown rust developed



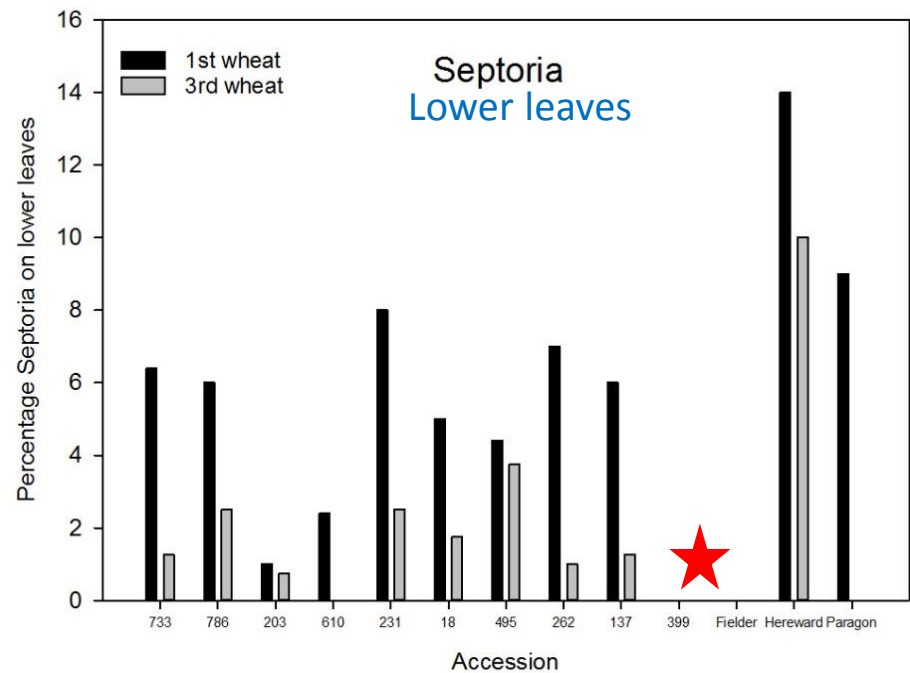
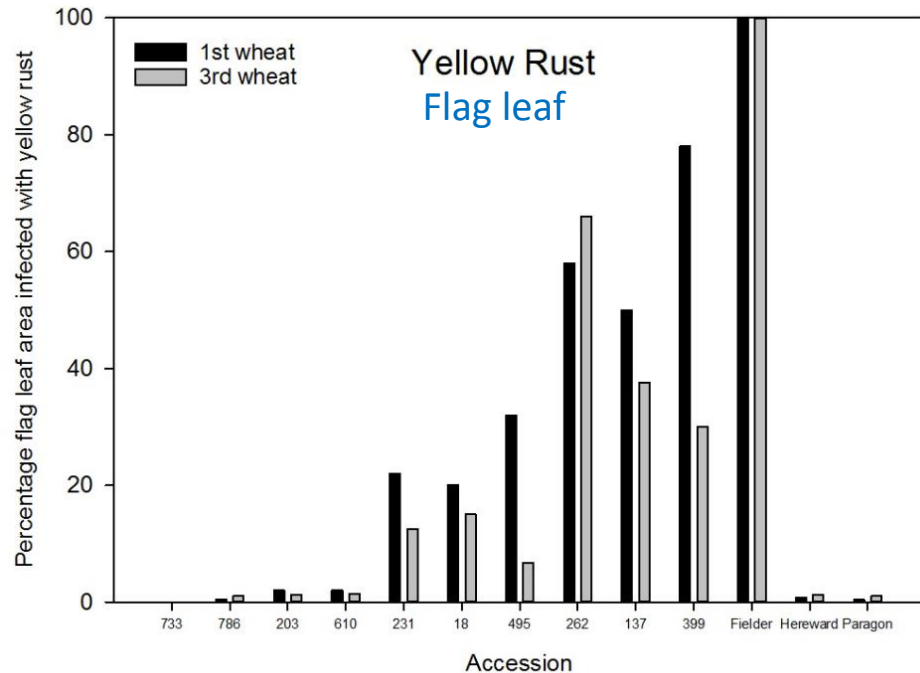


# 6<sup>th</sup> June 2016 – Yellow rust and Septoria



SCOTT WATSON

4



- Yellow Rust shows similar pattern to 2015

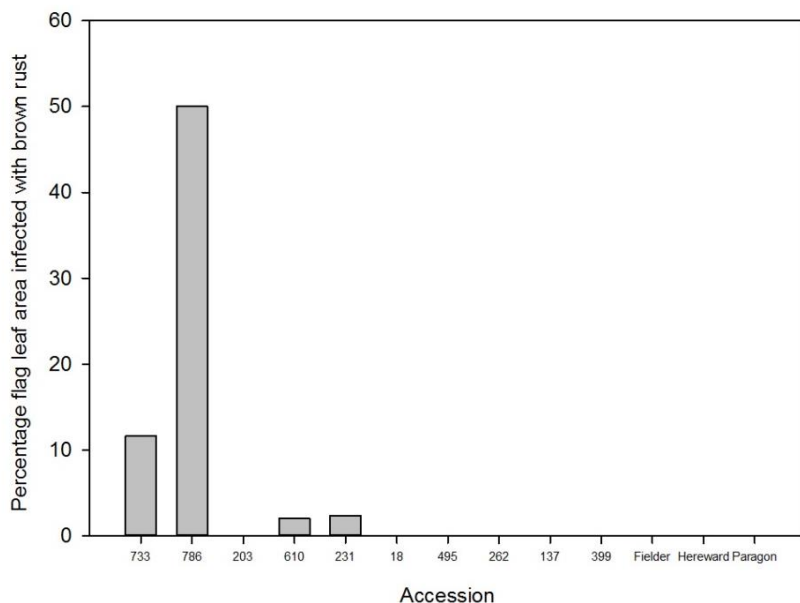
★ Low levels of Septoria on all genotypes except 399 and Fielder where high levels of yellow rust are found, therefore impossible to assess Septoria infections

- All genotypes at GS 57-61, except 610 at GS 47

# 22<sup>nd</sup> June 2016 – Brown rust developed across the 1<sup>st</sup> wheat trial site



ROTHAMSTED  
RESEARCH



Watkins 786



Watkins 733

- Watkins 733 and 786 were most resistant to yellow rust but are very susceptible to brown rust – do not possess multi disease resistance
- Watkins 203 most promising for showing high levels of resistance against both yellow rust, brown rust and septoria

# Watkins mapping population development



ROTHAMSTED  
RESEARCH

## Summer 2015 Field crossing with cv. Fielder

Accession Number	Growth habit	Country of Origin	Ears crossed	F <sub>1</sub> grains
203	Winter	India	8	31
231	Spring	Hungary	8	54
610	Spring	Yugoslavia	6	33
733	Spring	Iran	6	49
786	Spring	USSR	N/A	N/A

## Summer 2016

- F<sub>1</sub> grain sown in glasshouse to generate F<sub>2</sub> and for backcrossing to cv. Fielder
- Watkins 786 x Fielder crossing carried out in glasshouse
- F<sub>1</sub> grain included in a spring field trial 2016 to study inheritance

# F<sub>1</sub> plants – spring field trial



ROTHAMSTED  
RESEARCH

- Six F<sub>1</sub> grain from each of the 4 crosses sown
- Two replicate plots of parent genotypes (40 seeds per plot)
- Yellow Rust assessments on 30<sup>th</sup> June 2016

Parents		Fielder x 203	
Fielder	203	Plant 1	Plant 2
100%	2.5%	50%	50%

Parents		Fielder x 231
Fielder	231	Plant 1
100%	5%	30%

Parents		Fielder x 610	
Fielder	610	Plant 1	Plant 2
100%	7.5%	50%	70%

Parents		Fielder x 733				
Fielder	733	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
100%	0%	0%	trace	0%	0%	0%

# 2016-2017 field trials



ROTHAMSTED  
RESEARCH

## F<sub>2</sub> field trial

- Two F<sub>2</sub> populations from each of the four crosses sown (FxW203, FxW231, FxW610 and FxW733) + parents
- 10 F<sub>1</sub> plants sown from FxW786 cross
- Plot size = 4 rows x 1 m length, 80 seeds sown for each population

## Watkins multi-disease resistance

- 3<sup>rd</sup> repeat sown of 10 Watkins lines + controls in 1<sup>st</sup> and 3<sup>rd</sup> wheat fields

# Summary



ROTHAMSTED  
RESEARCH

- Five Watkins accessions with evidence of moderate-high resistance against YR
- Watkins 733 – extreme YR resistance dominant
- Watkins 203, 231, 610 – YR resistance appears to be recessive
- Watkins 203 most promising accession for multi-disease resistance
- Trend towards less foliar disease in 3<sup>rd</sup> wheat field trial across both seasons

## 2016-2017 field trials

- Repeat 1<sup>st</sup> and 3<sup>rd</sup> wheat multi-disease resistance trials
- F<sub>2</sub> inheritance trial
- MDR031 (R) x MDR043 (S) take-all phenotyping trial



# Many thanks to

---



ROTHAMSTED  
RESEARCH

Kim Hammond-Kosack

## PhD students

Sarah-Jane Osborne

Joseph Moughan

## Undergraduate summer students

Erin Baggs

Eleanor Leane

Tessa Reid

Laurie Neal

Alex Chambers-Ostler

Leanne Freeman

Gail Canning - seed preparation

Mike-Hammond-Kosack - crossing

Rodger White - statistics

RRes farm and glasshouse staff