JIC Thermal imaging 2016, 2017 & 2018

Part 1:

Andrew Riche

John Innes Centre
Unlocking Nature's Diversity

ROTHAMSTED RESEARCH

Department for Environment Food & Rural Affairs

Wheat Genetic Improvement Network

BBSRC bioscience for the future

Lawes Agricultural Trust

Background

- Paragon x Garcia mapping population, 177 lines + parents
- 9 Paragon library NILs also included
- Soissons used at edges and within expt. 44 plots, both irrigated and unirrigated.
- JIC Church Farm, Bawburgh, nr Norwich

ROTHAMSTED RESEARCH

Methodology

	25/05/17 (8:47-15:33 GMT)		05/07/18 (9:25-15:44 GMT)	
Flight	Start Time	Interval (min)	Start Time	Interval (min)
1	08:47		8:56	Failed
2	9:38	50	9:25	
3	10:40	62	10:26	61
4	11:14	34	11:27	61
5	11:58	44	12:40	73
6	13:16	78	13:26	46
7	14:03	46	14:31	65
8	(14:46 Data lost)	42	15:11	40
9	15:33	48	15:44	33

Also 1 RGB+NIR flight on each date

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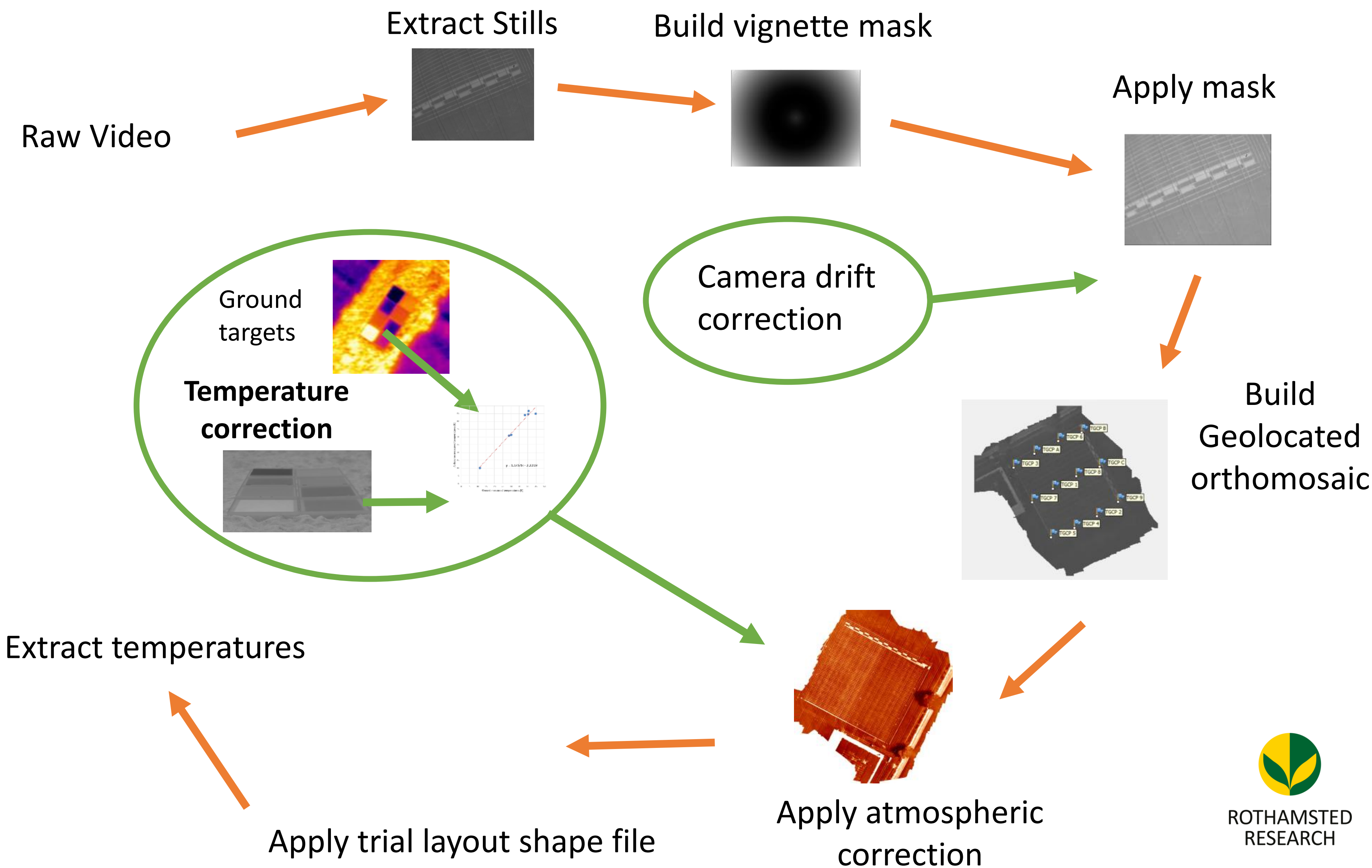
Thermal image processing



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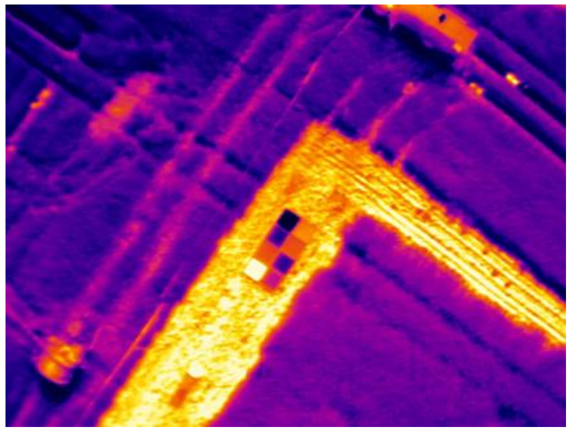
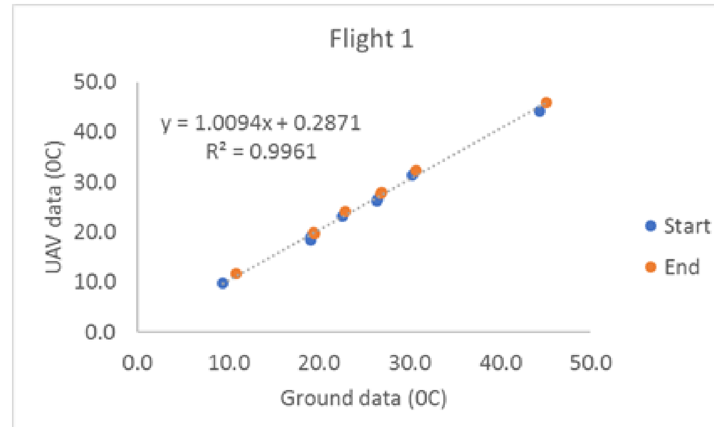
TIR – Atmospheric correction



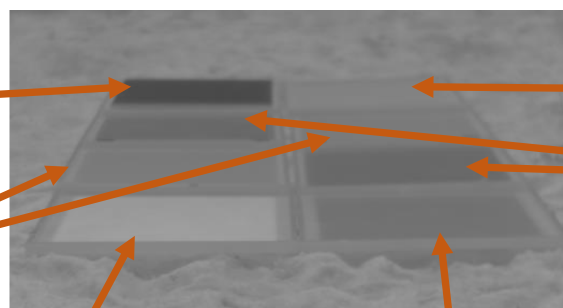


Polystyrene

Bare Wood

White Card

Grey Card



Black paint

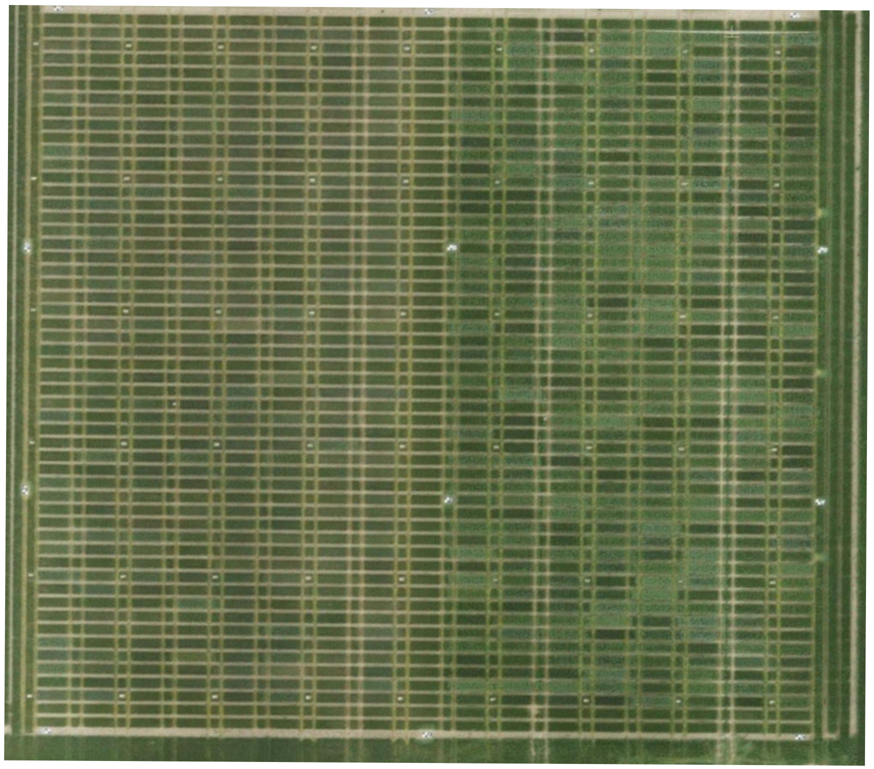
White paint

ROTHAMSTED RESEARCH

Drought trial Thermal imaging

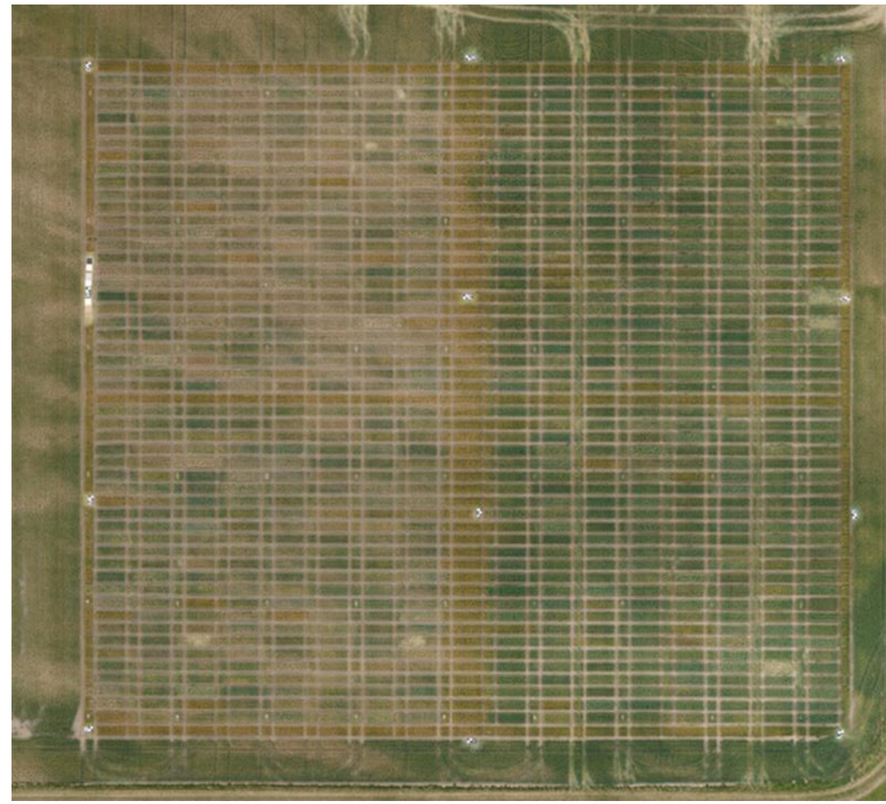
2017

Rain fed Irrigated



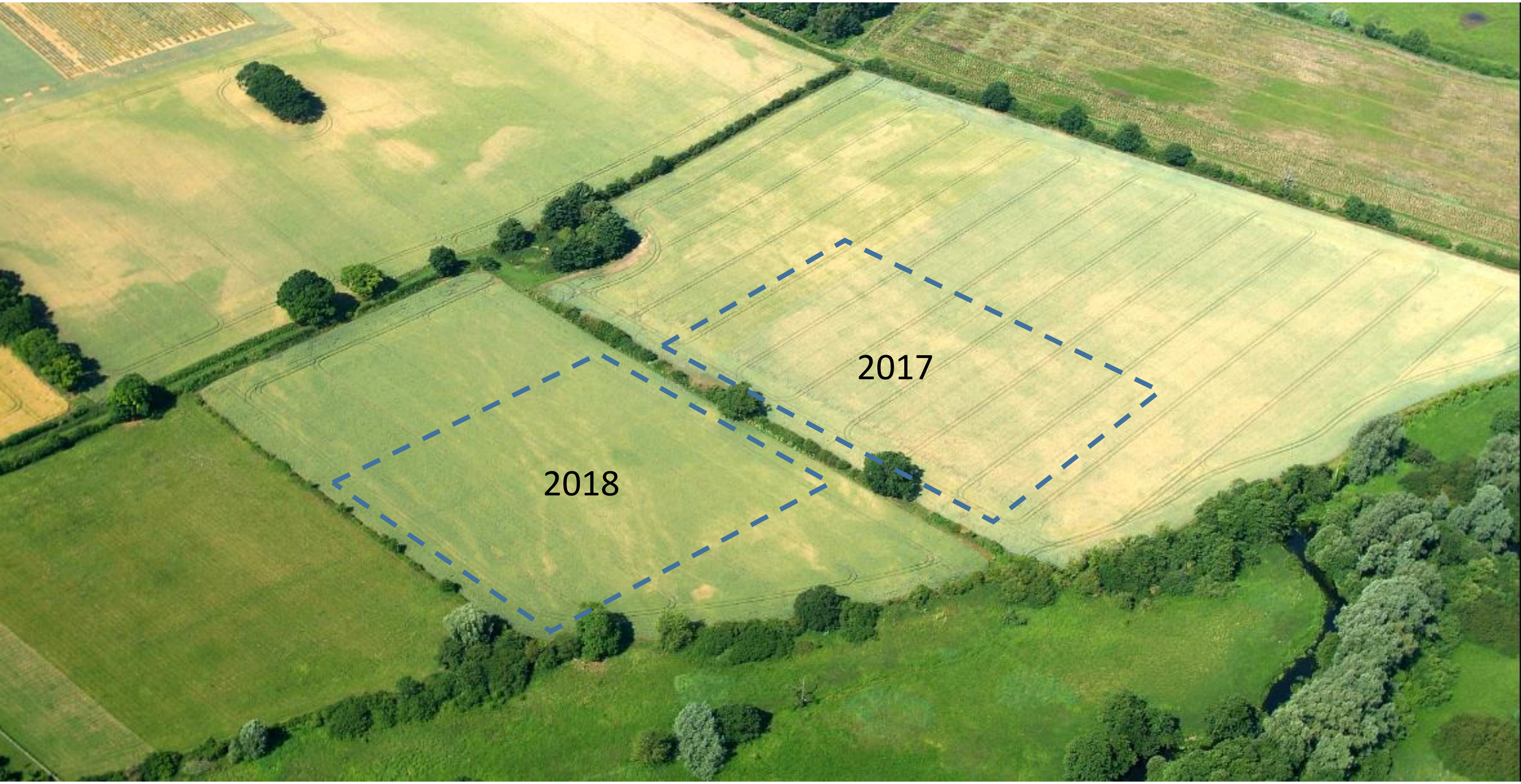
2018

Rain fed Irrigated



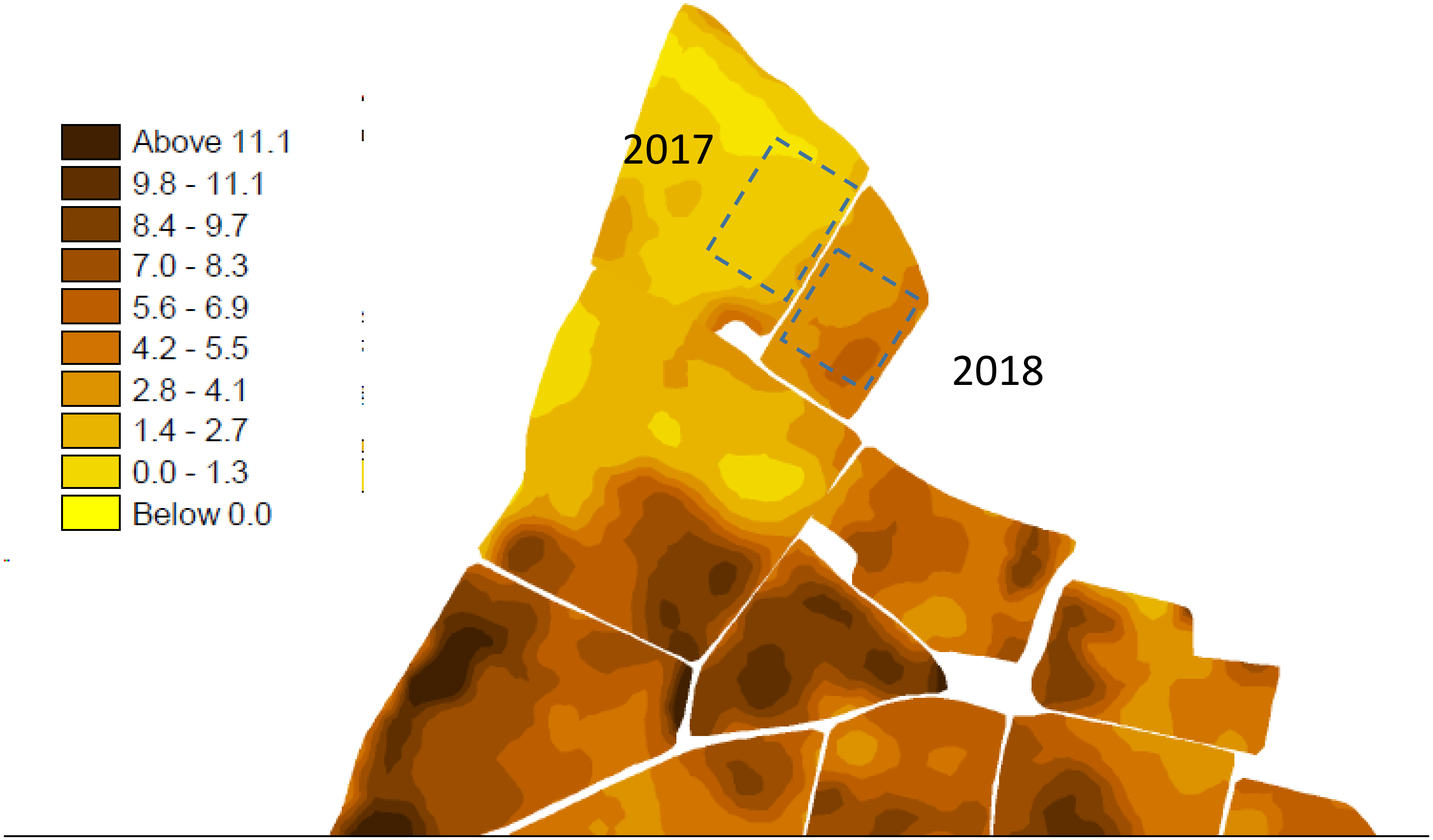
ROTHAMSTED
RESEARCH

Approximate locations



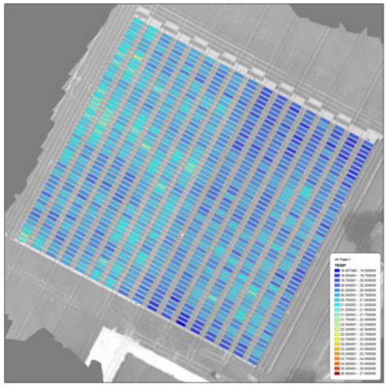
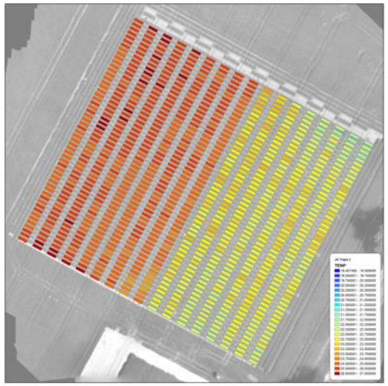
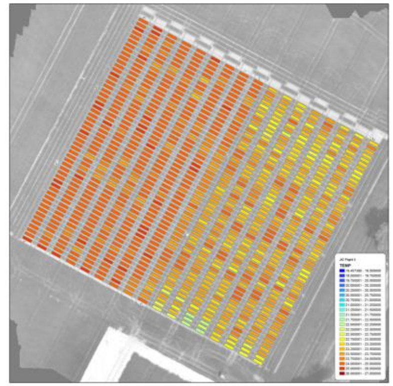
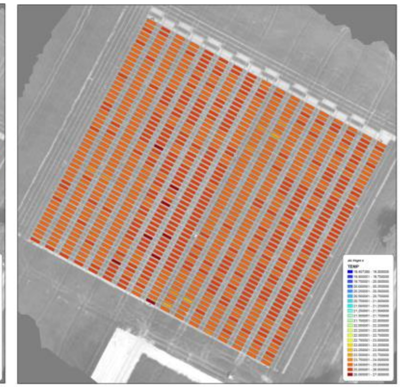
ROTHAMSTED RESEARCH

Shallow EC Oct 2013 0-40cm

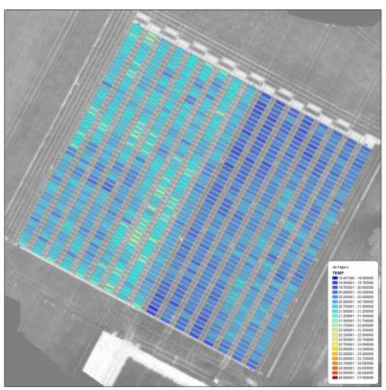
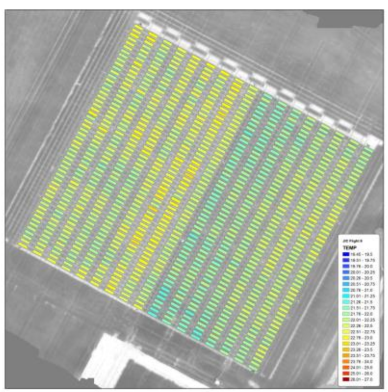
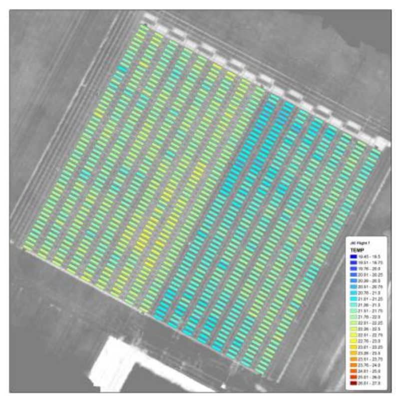
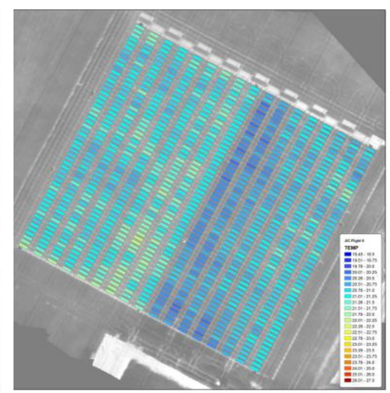


ROTHAMSTED RESEARCH

Results 2017 (Time GMT)

8:47, 24.7^{0}C (air temp) 9:38, 27.5^{0}C 10:40, 28.2^{0}C 11:14, 29.7^{0}C

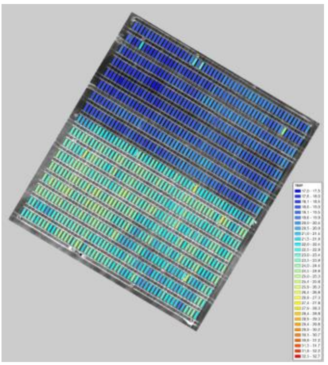
11:58, 24.5^{0}C 13:16, 27.9^{0}C 14:03, 28.9^{0}C 15:33, 26.5^{0}C

TEMP
19.45 - 19.5
19.51 - 19.75
19.76 - 20.0
20.01 - 20.25
20.26 - 20.5
20.51 - 20.75
20.76 - 21.0
21.01 - 21.25
21.26 - 21.5
21.51 - 21.75
21.76 - 22.0
22.01 - 22.25
22.26 - 22.5
22.51 - 22.75
22.76 - 23.0
23.01 - 23.25
23.26 - 23.5
23.51 - 23.75
23.76 - 24.0
24.01 - 25.0
25.01 - 26.0
26.01 - 27.0

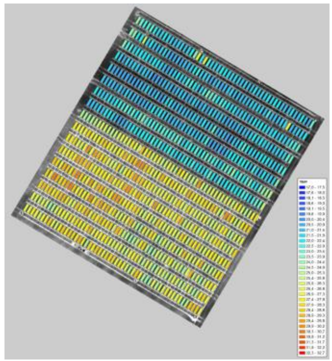
Irrigated side heats up more slowly, then cools quicker than the rain-fed.

ROTHAMSTED RESEARCH

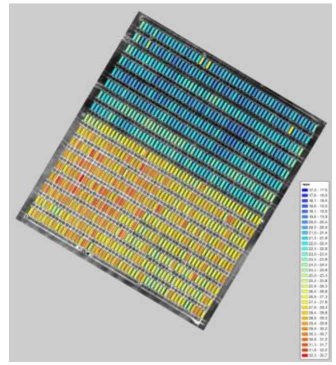
Results 2018 (Time GMT)



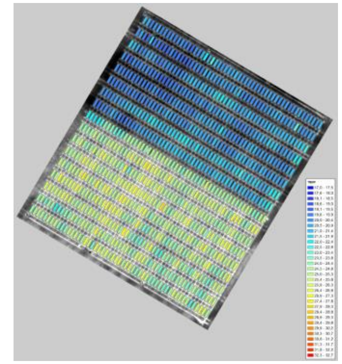
9:23, 27.4^{0}C (air temp)



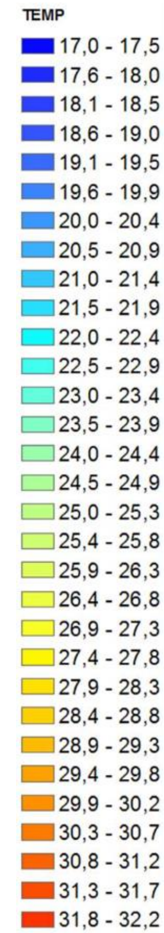
10:24, 26.7^{0}C

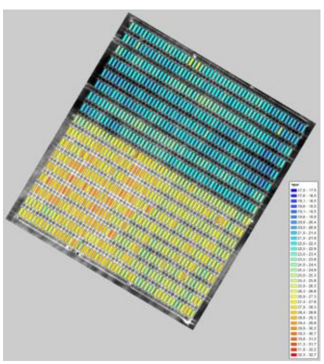


11:26, 28.7^{0}C

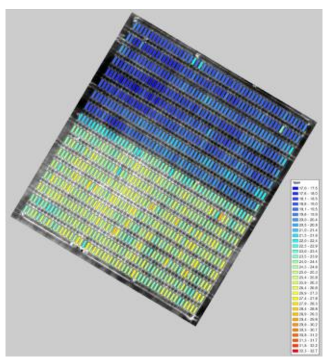


12:39, 28.8^{0}C

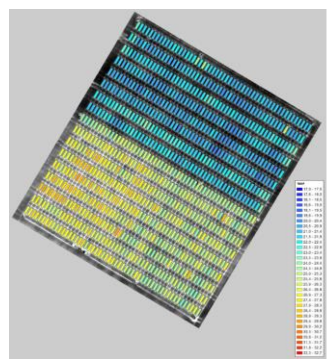




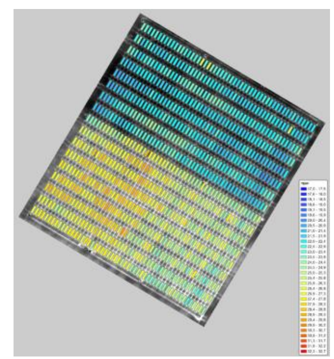
13:24, 29.2^{0}C



14:29, 31.6^{0}C



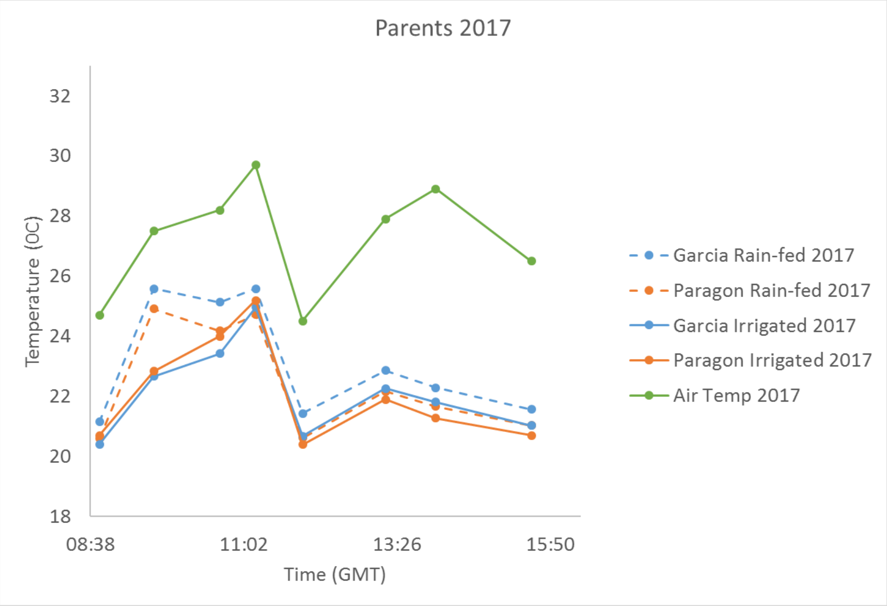
15:09, 28.5^{0}C

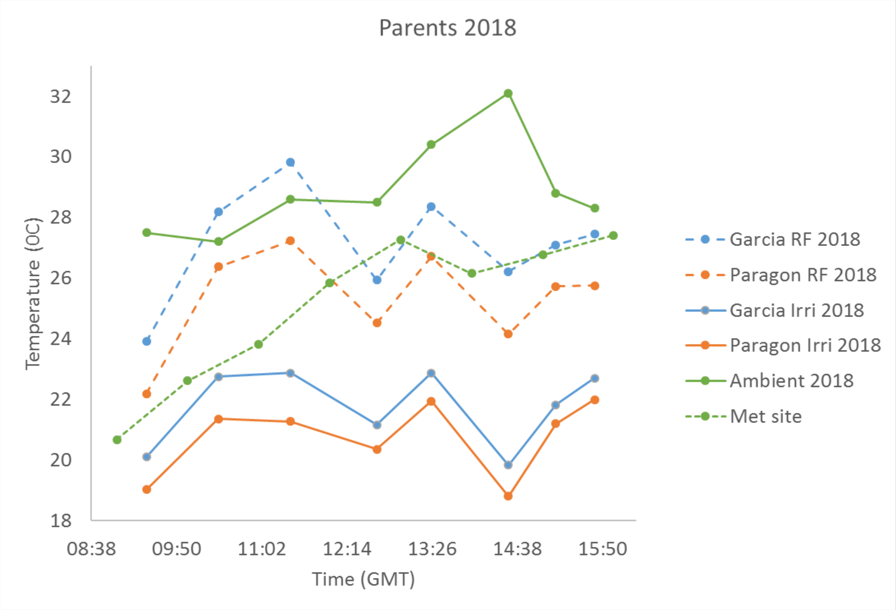


15:42, 28.0^{0}C

ROTHAMSTED RESEARCH

Canopy Temperature





Canopy temperature tracked air temperature

2017:

- Ambient temperature 25 to 30^0
- Rainfed plots higher temperature than irrigated (about 1degree)

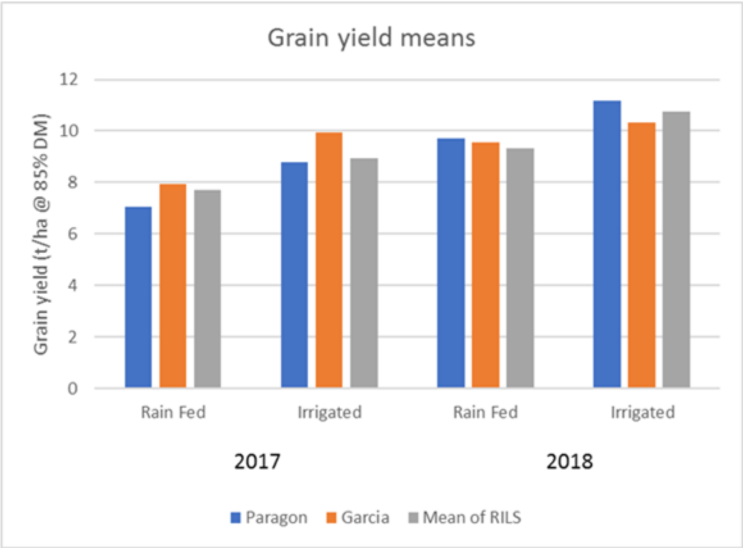
2018:

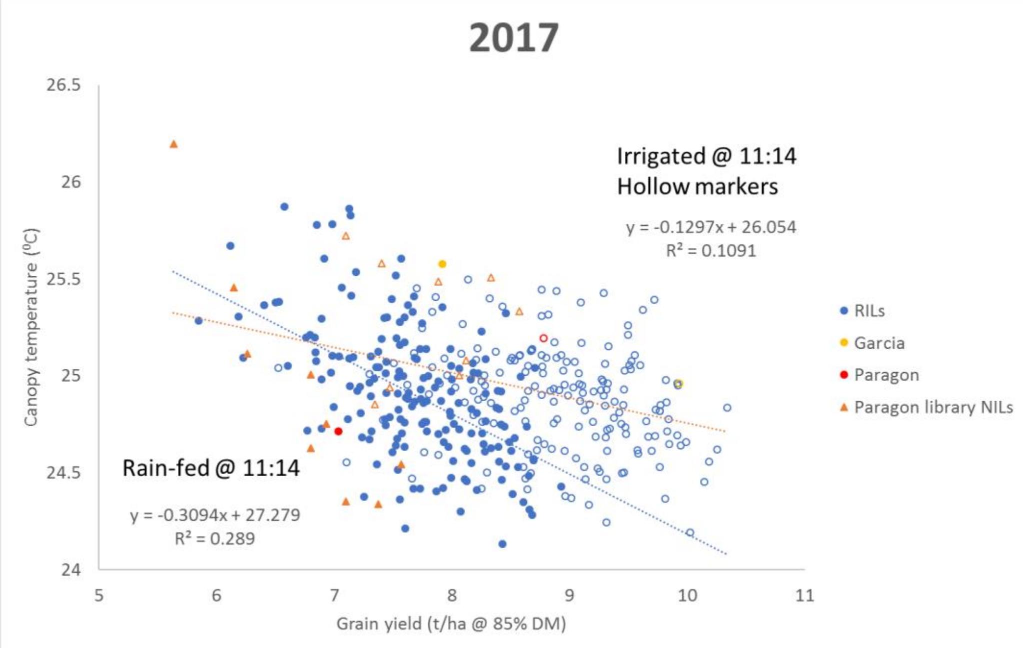
- Rain-fed plot's temperature closer to ambient than 2017
- Garcia higher than Paragon
- Several irrigated lines had a lower temp at 14:24 than at 9:25
- Ambient temperature 27 to 32^0

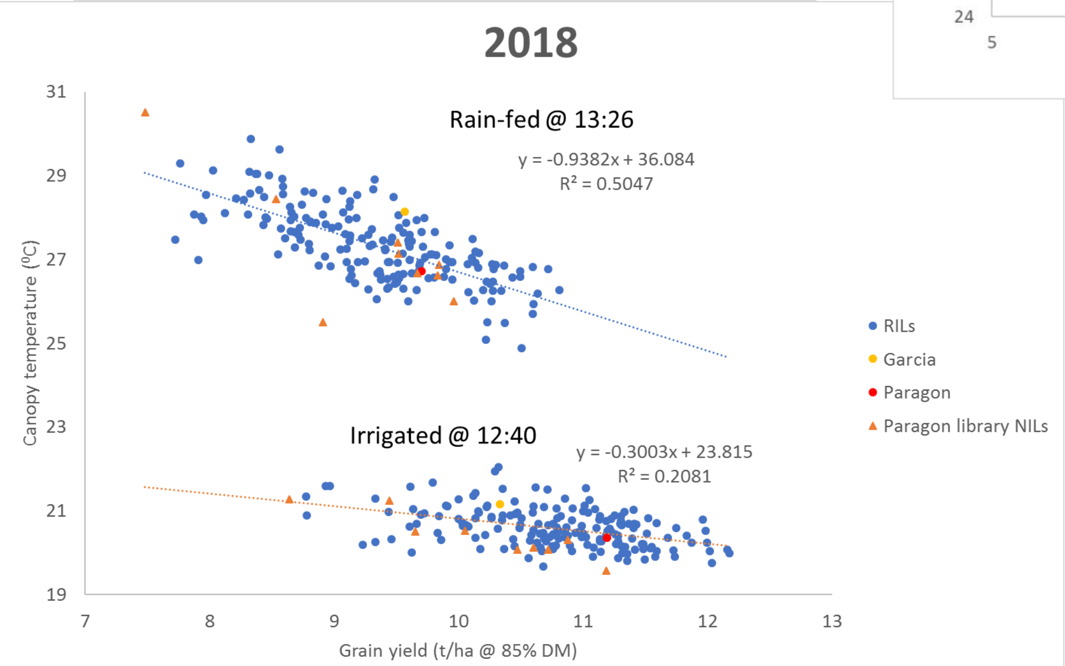
- Both yrs, at about 13:30, ambient temperature increased but canopy temperature decreased – greater resolution of ambient temperature may discount this

ROTHAMSTED RESEARCH

Yield & Canopy temperature



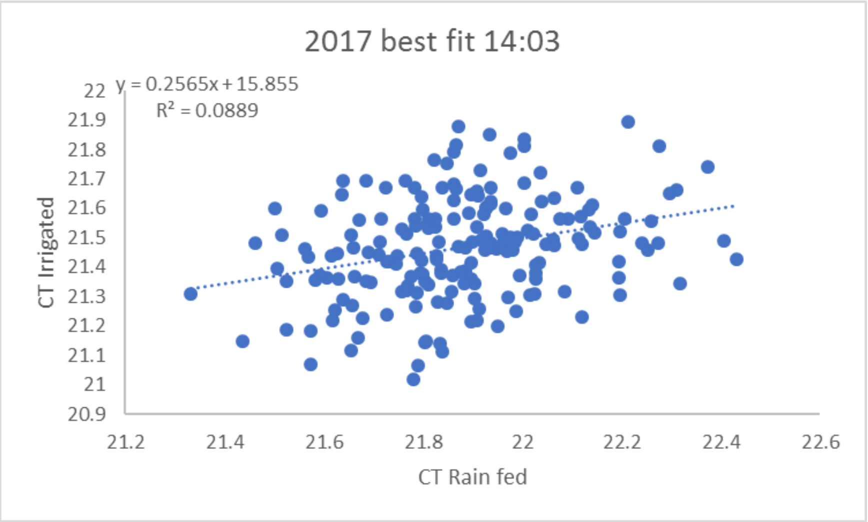


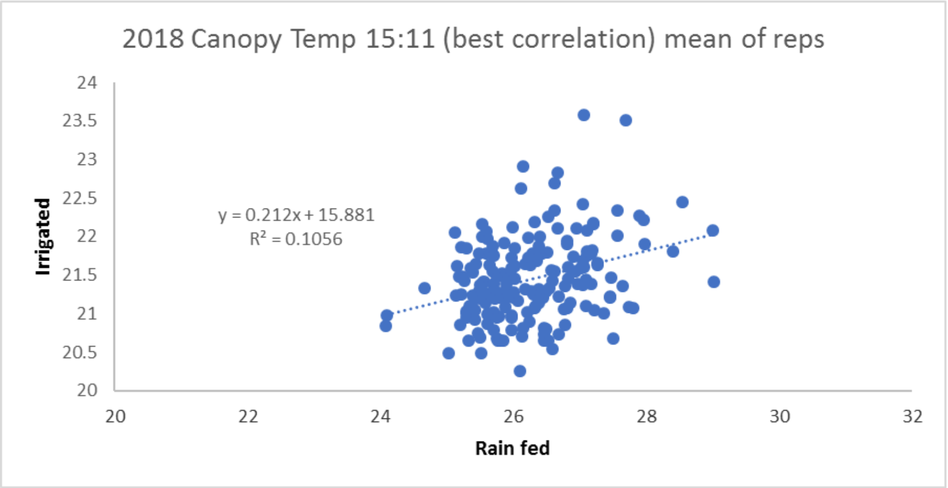


ROTHAMSTED RESEARCH

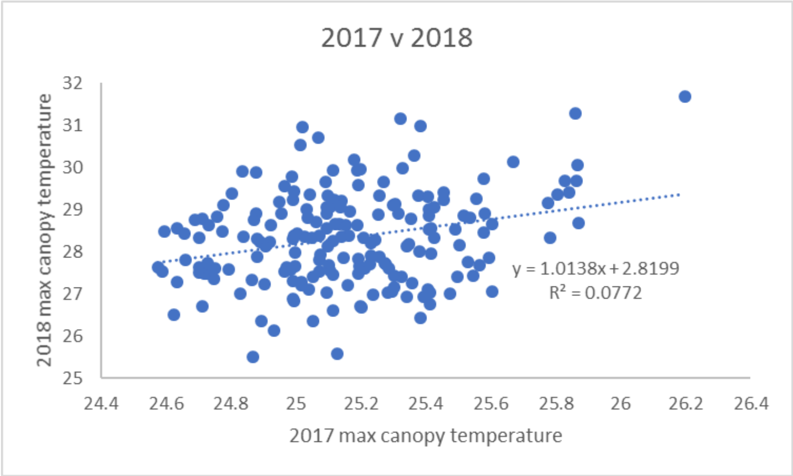
Results

Within year correlations

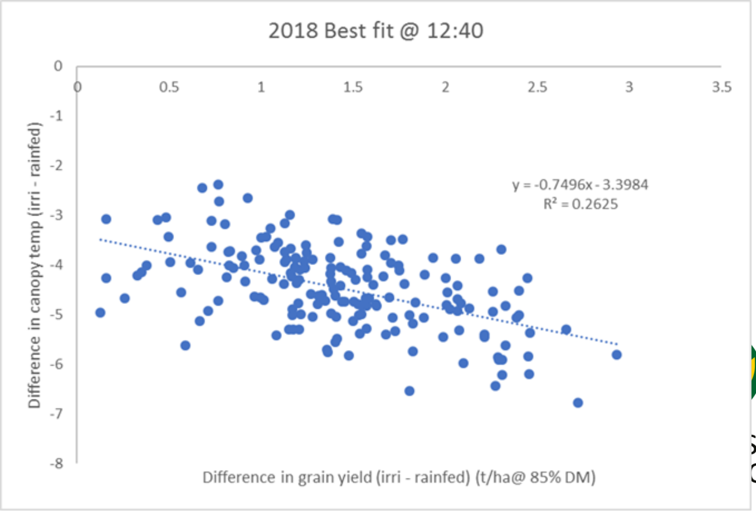




Between years:



Effect of irrigation:



Acknowledgments

Rothamsted
Malcolm Hawkesford
March Castle
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ROTHAMSTED RESEARCH

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Adam Michalski

WROCŁAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES

JIC
Simon Griffiths
Clare Lister
Cathy Mumford
Simon Orford

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Wheat Genetic Improvement Network

BBSRC bioscience for the future

Paragon x Garcia Drought Trials

WGIN Management Meeting

14/2/2019

Clare Lister and Simon Griffiths, JIC

Andrew Riche, Malcolm Hawkesford and the Drone Team, Rothamsted

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ROTHAMSTED RESEARCH

Introduction

- Aim was to look at the effect of spring drought
- 3 x Trials 2016/2017/2018
- 177 PxG RILs – enriched for *Ppd*-sensitive (from total population size = 356)
- Nine Paragon Library lines
- Paragon and Garcia controls, plus Soisson
- 2 reps – Irrigated (IR)
- 2 reps – Not Irrigated (NI) (= Rain-fed)
- 800 plots in total each trial
- Usual field phenotyping (stage 31, booting, flowering, height)
- Harvest and post-harvest measurements (YLD, SW, TGWT, grains / m^2)
- Soon to do NIR
- QTL mapping
- Rothamsted (and JIC) drone

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Trial Layout and Lines

NI

157	94	191	45	91	95	84	13	39	7	5	26	140	134	46	25	133	136	124	2	37	63	162	155	175
146	23	135	100	199	109	4	86	54	38	198	122	19	174	29	34	161	85	182	177	104	195	160	76	10
200	176	111	168	1	12	165	156	9	87	144	179	193	52	141	75	35	77	166	22	145	151	81	148	61
152	125	110	149	127	40	96	187	118	53	98	11	119	50	49	147	113	117	190	72	142	159	116	186	93
57	33	42	14	74	79	65	56	43	164	55	58	32	121	62	59	139	66	102	70	171	3	189	188	128
194	47	184	180	172	36	129	196	20	112	99	73	114	163	24	197	120	103	173	123	90	169	44	64	183
88	17	30	69	158	107	132	185	192	126	137	21	167	97	16	108	48	92	178	181	68	106	27	60	78
89	8	83	150	143	130	101	153	154	31	82	105	15	18	170	115	131	138	28	6	67	41	71	51	80

151	119	152	36	142	196	3	104	72	150	58	186	122	83	158	187	108	93	163	182	16	154	75	99	88
162	60	179	98	61	171	134	2	193	35	5	63	18	41	69	166	155	105	153	74	132	64	94	113	140
103	92	133	97	107	10	44	6	50	56	96	101	188	32	185	85	24	14	160	131	20	143	124	195	73
4	66	197	80	146	106	91	51	168	84	37	138	175	47	198	194	180	169	7	139	86	31	78	148	1
189	67	184	125	40	173	147	161	82	127	172	70	181	21	109	89	53	12	52	170	174	183	129	34	123
27	9	87	8	68	136	11	199	145	126	55	62	141	137	128	57	77	54	71	26	22	115	15	167	25
176	164	117	116	177	190	45	159	79	13	157	95	42	90	59	110	33	200	28	192	156	29	65	191	130
81	43	149	165	30	19	46	121	100	112	38	120	102	76	23	111	39	178	118	114	17	49	135	144	48

IR

114	158	31	107	86	32	67	95	149	159	153	173	165	46	22	20	18	195	134	129	169	50	40	118	126
52	190	102	197	155	51	174	97	143	15	110	138	10	127	43	184	68	112	121	74	177	13	198	7	58
16	188	65	66	140	186	78	14	175	105	196	17	131	37	180	194	98	145	70	19	55	144	156	30	9
60	106	91	1	79	39	193	3	27	116	171	151	41	99	44	73	109	148	24	136	53	191	47	179	111
35	199	21	132	164	33	75	103	187	181	64	62	28	63	152	48	93	56	137	69	84	5	150	59	76
176	185	87	160	113	125	8	80	178	133	36	130	6	115	182	139	200	146	162	71	161	88	167	183	135
82	72	2	54	26	77	42	122	128	124	11	172	85	166	92	89	90	168	170	101	25	119	100	192	38
94	142	141	4	83	57	104	34	147	108	189	12	123	120	96	154	45	49	23	61	157	81	117	163	29

16	88	3	124	138	110	59	144	198	185	41	29	116	72	13	155	75	156	63	4	47	97	176	132	71
105	64	153	142	17	154	118	27	104	14	80	65	67	160	193	76	42	172	66	200	122	101	96	36	46
163	192	186	10	35	174	128	44	86	100	191	18	109	25	161	54	150	147	135	15	50	190	6	149	60
134	162	58	141	166	57	175	108	92	87	49	125	113	165	20	5	112	98	12	40	139	94	69	158	38
197	107	130	180	117	179	194	119	173	73	120	143	114	26	2	79	32	31	68	115	43	82	136	91	188
99	37	177	182	168	53	51	164	55	159	184	19	131	7	74	196	121	123	95	181	84	77	90	195	103
129	22	39	145	157	133	111	102	171	23	183	61	45	62	81	83	34	189	21	126	28	56	11	30	169
148	93	187	170	199	89	137	106	146	33	70	127	152	24	167	140	151	52	9	48	78	85	1	178	8

SLOPE

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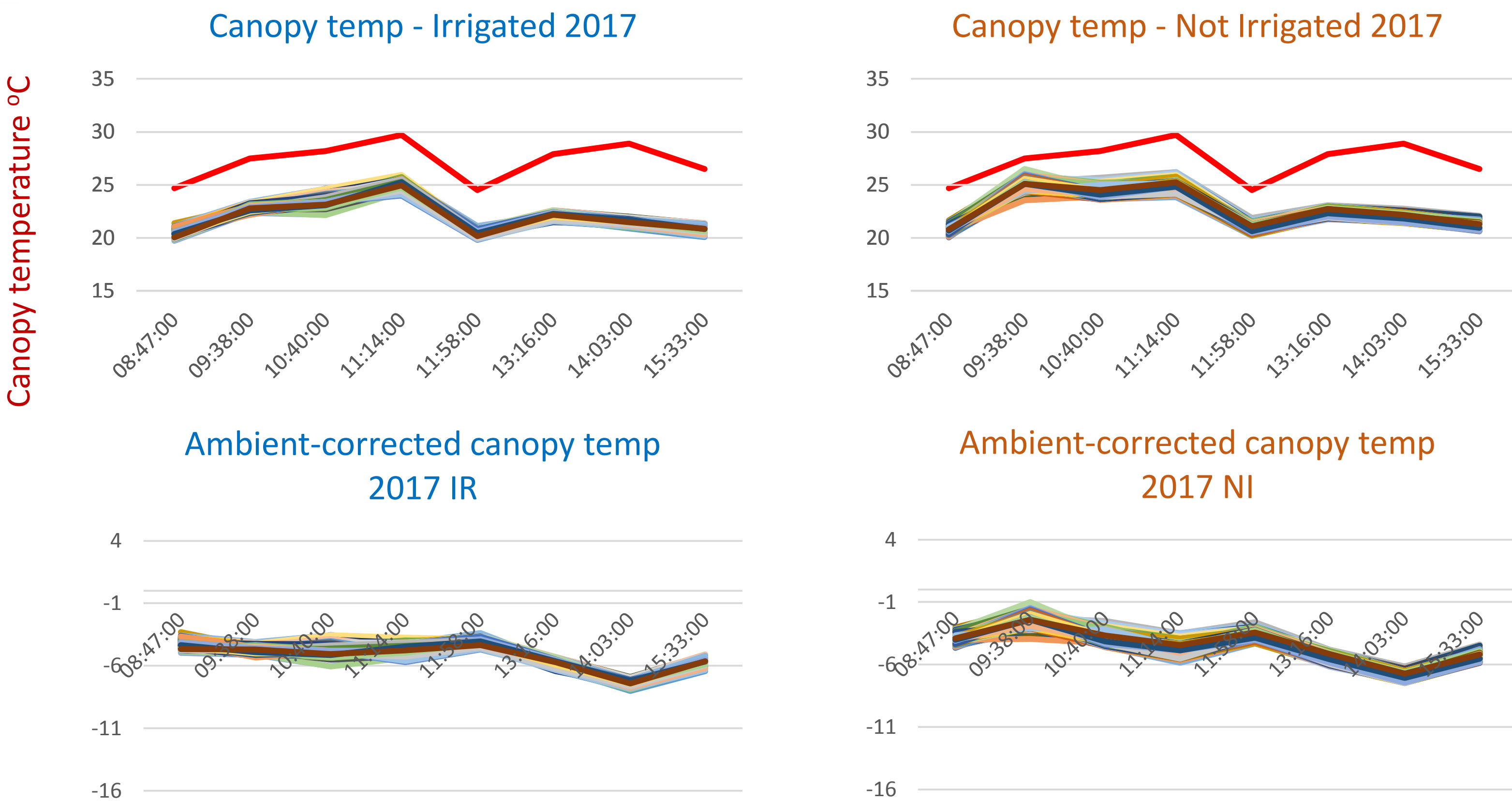
ROTHAMSTED RESEARCH

Canopy Temperature 28/5/2017



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Canopy Temperature 5/7/2018

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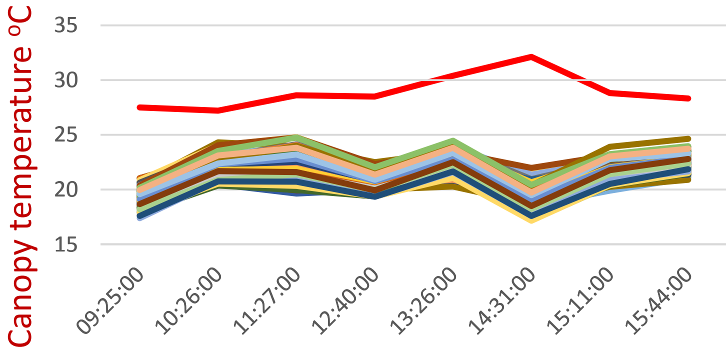
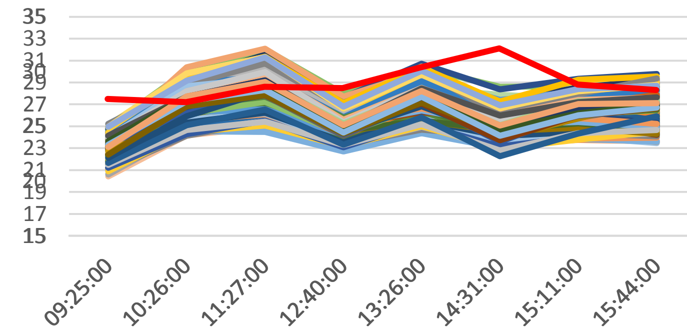
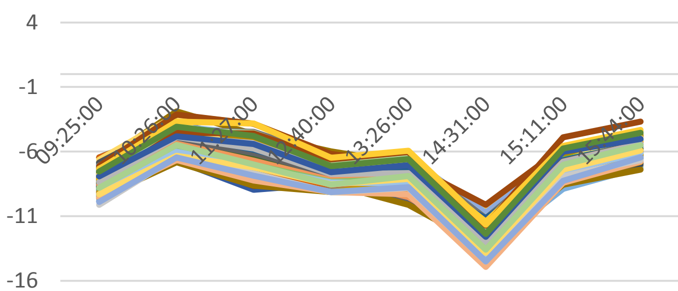
Canopy temp - Irrigated 2018



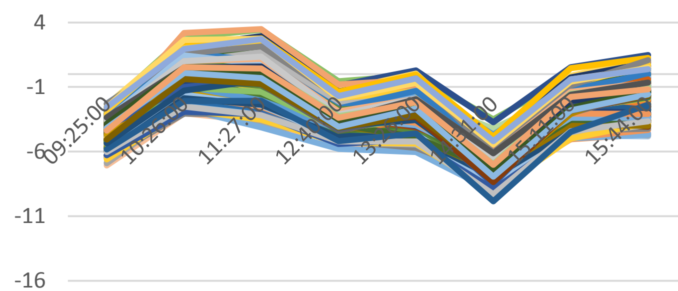
Canopy temp - Not Irrigated 2018



Ambient-corrected canopy temp 2018 IR



Ambient-corrected canopy temp 2018 NI



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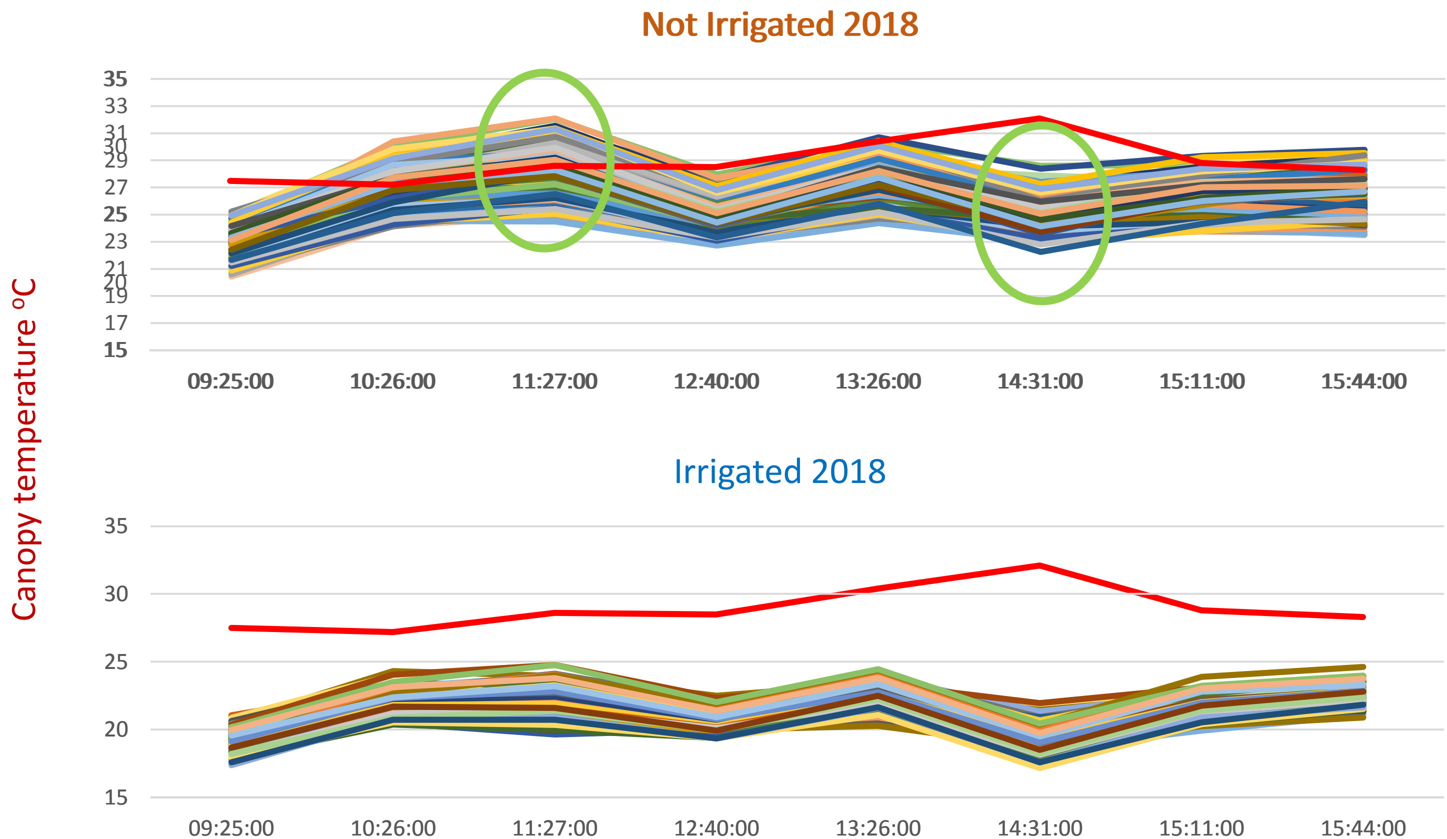
Canopy Temperature 5/7/2018



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QTL mapping Canopy Temp @ 11.27

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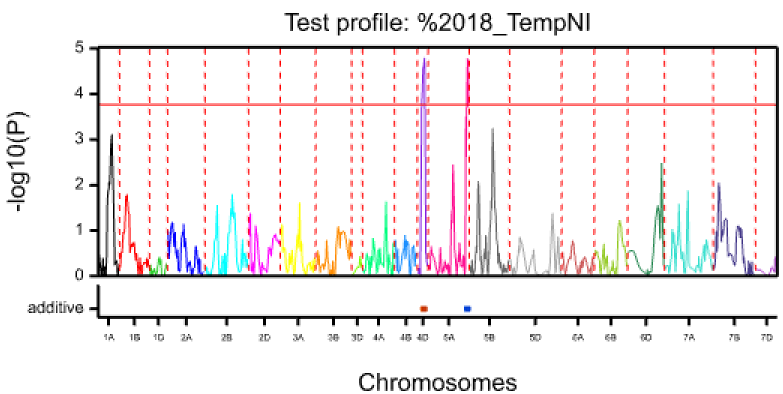
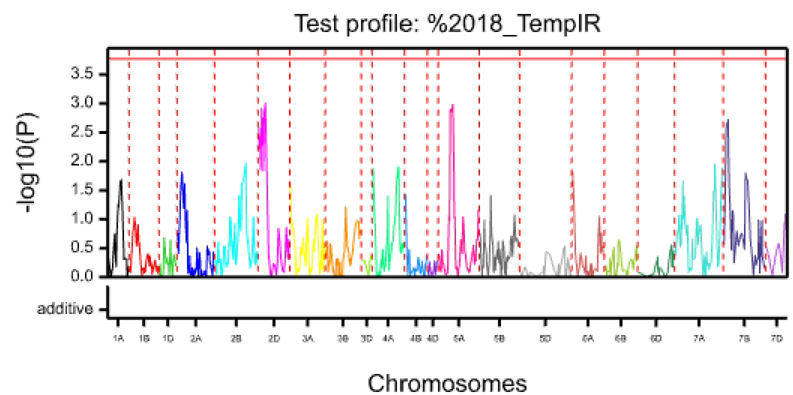
Mean of Not-Irrigated plots in 11.27 flight
2 QTLs (above threshold)

Mean of Irrigated plots in 11.27 flight
NO QTLs (above threshold)



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



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

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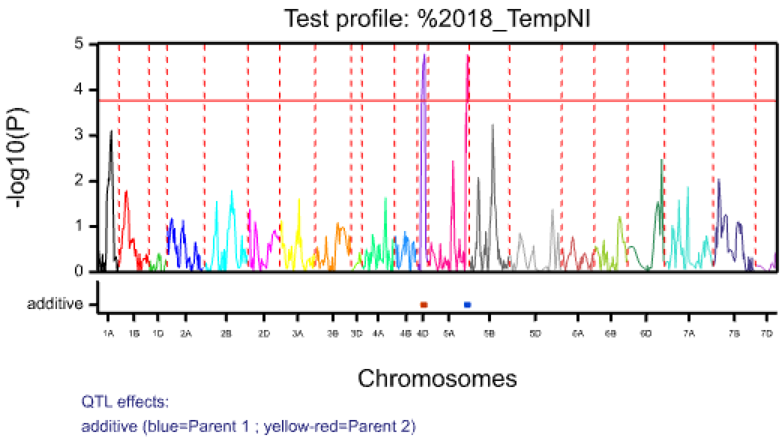
QTL mapping Canopy Temp @ 11.27

Mean of Not-Irrigated plots in 11.27 flight – 2 QTLs



Trait: %2018_TempNI

List of QTLs

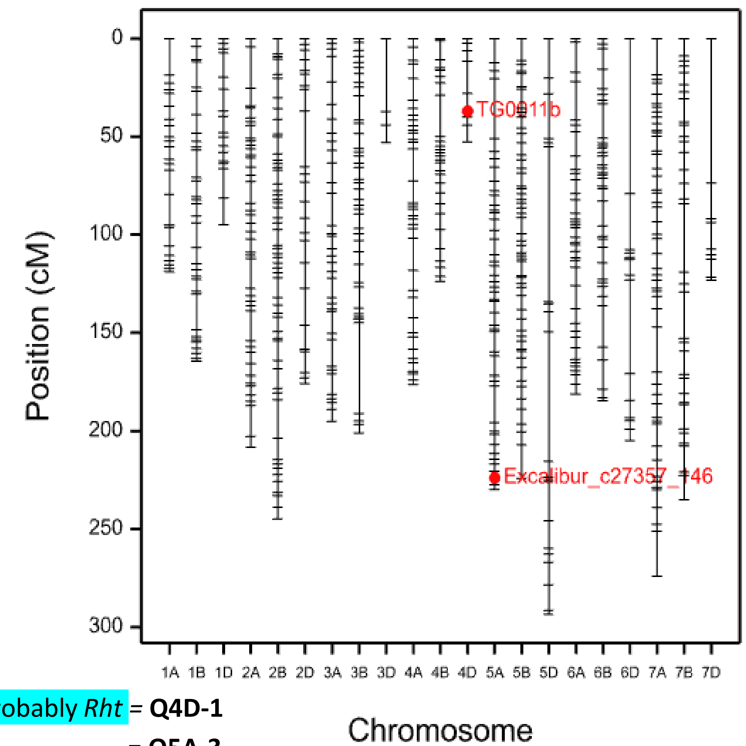
Locus no.	Locus name	Linkage group	Position	-log10(P)
401	TG0011b	4D	37.06	4.402
454	Excalibur_c27357_146	5A	223.86	4.797

QTL effects

Locus no.	Locus name	%Expl. Var.	Add. eff.	High value allele	s.e.
401	TG0011b	10.018	0.337	Garcia	0.080
454	Excalibur_c27357_146	10.601	0.346	Paragon	0.078

Estimated lower and upper bounds of QTL positions

Locus no.	Locus name	Lower bound	Position	Upper bound	
401	TG0011b	29.246	37.060	44.874	
454	Excalibur_c27357_146	216.546	223.860	229.900	



Probably *Rht* = **Q4D-1**

= **Q5A-3**

Department for Environment Food & Rural Affairs

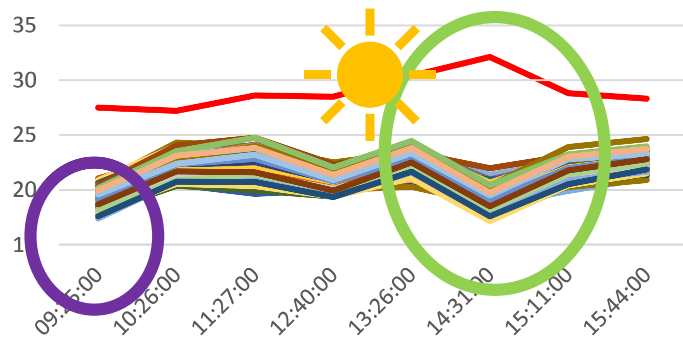
ROTHAMSTED RESEARCH

Wheat
Genetic
Improvement
Network

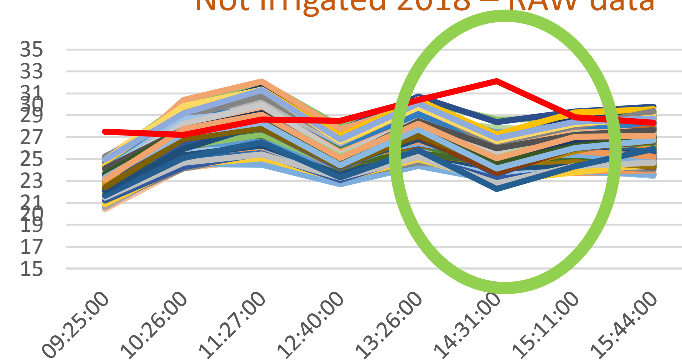
John Innes Centre

QTL mapping of the Canopy Temp Dip

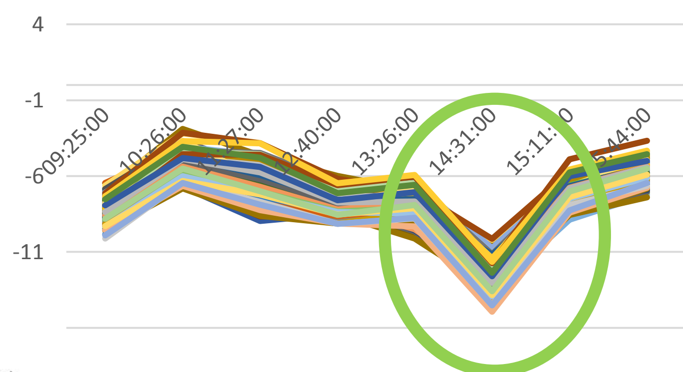
Irrigated 2018 - RAW data



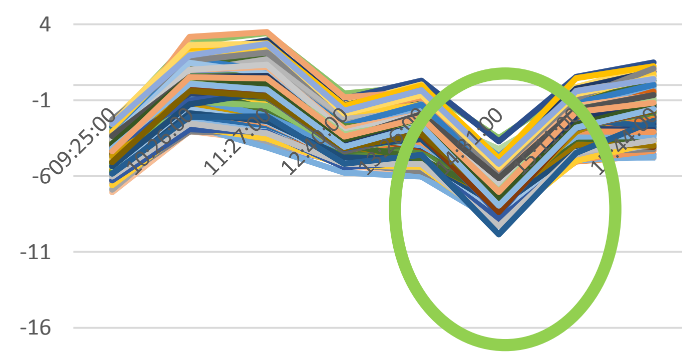
Not Irrigated 2018 – RAW data



Ambient-adjusted 2018 IR



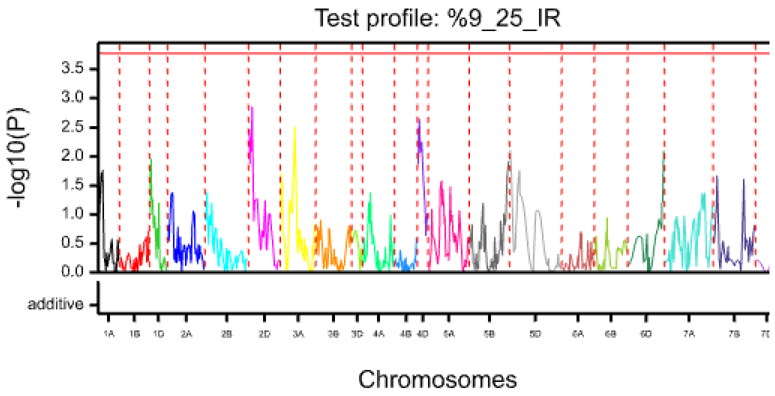
Ambient-adjusted 2018 NI

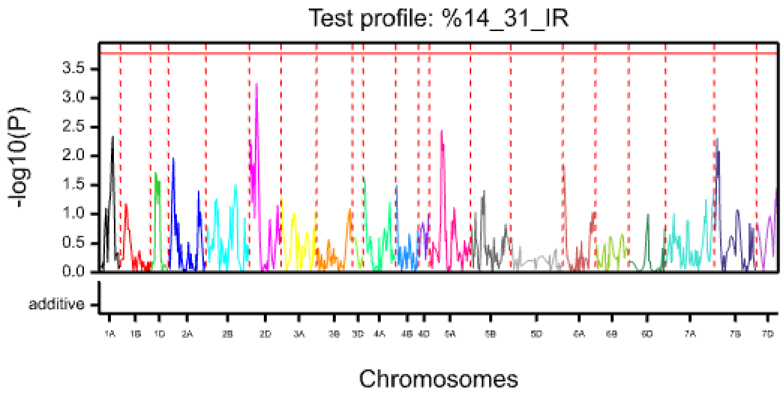


Department
for Environment
Food & Rural Affairs

ROTHAMSTED
RESEARCH

QTL mapping of Canopy Temp IR





Wheat Genetic Improvement Network

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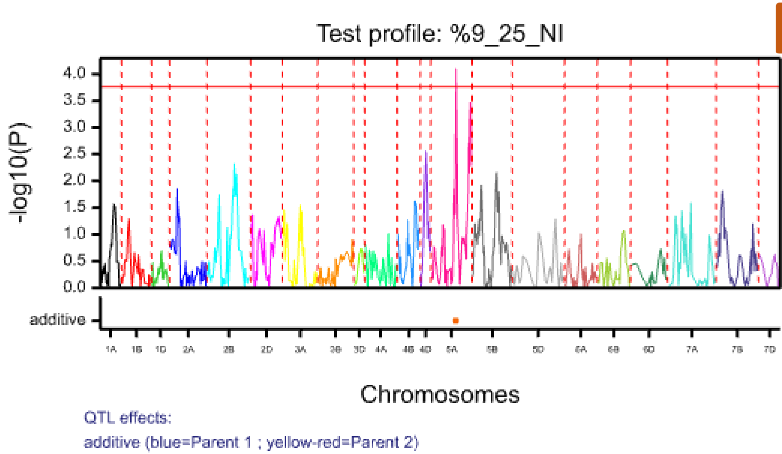
Department for Environment Food & Rural Affairs

ROTHAMSTED RESEARCH

QTL mapping of Canopy Temp @ 9.25 NI



Summary
Trait: %9_25_NI

List of QTLs

Locus no.	Locus name	Linkage group	Position	-log10(P)
435	BS00044408_51	5A	139.84	3.936

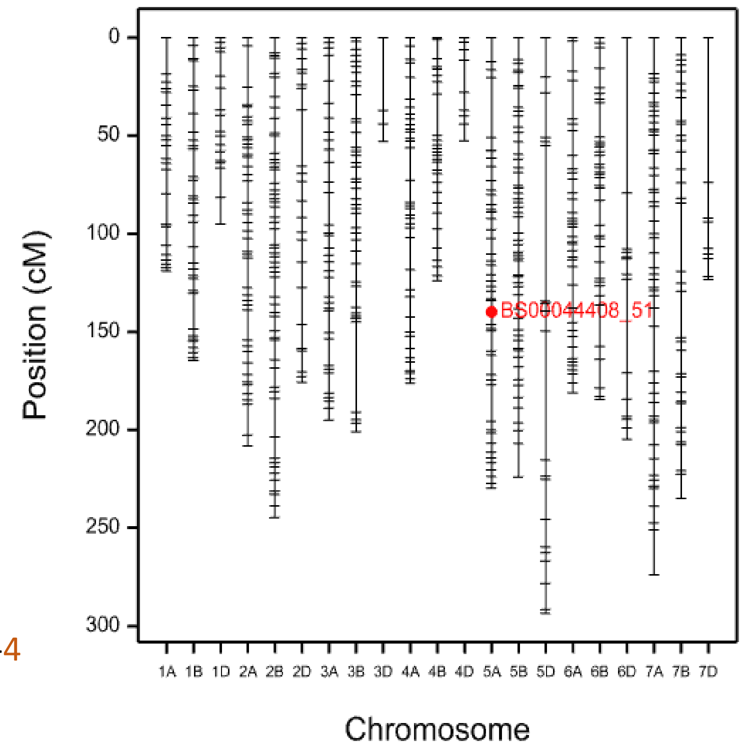
QTL effects

Locus no.	Locus name	%Expl. Var.	Add. eff.	High value allele	s.e.
435	BS00044408_51	9.093	0.194	Garcia	0.049

Estimated lower and upper bounds of QTL positions

Locus no.	Locus name	Lower bound	Position	Upper bound
435	BS00044408_51	131.076	139.840	148.604

=Q5A-4



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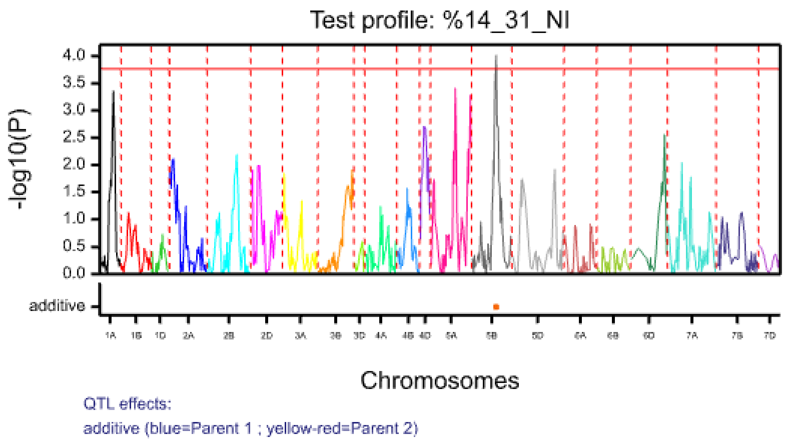
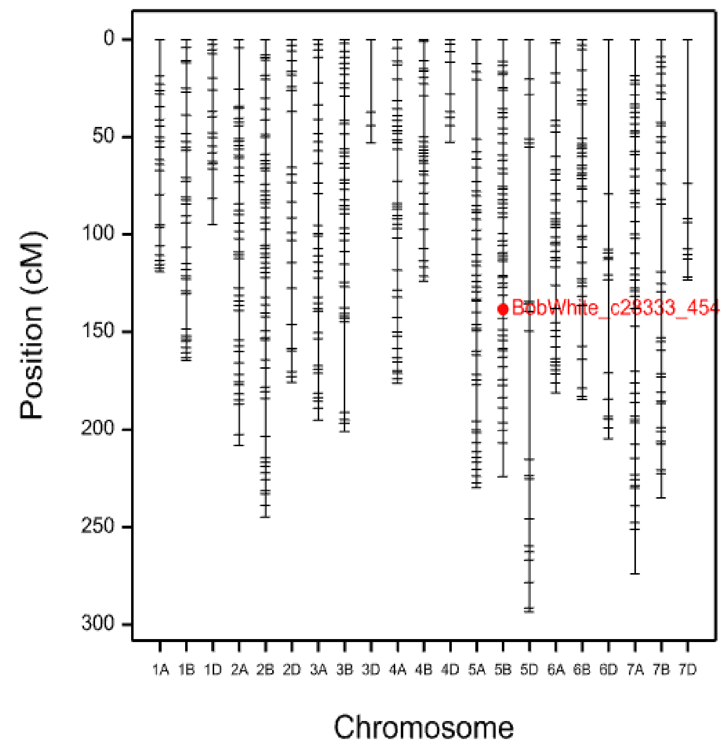
ROTHAMSTED RESEARCH

QTL mapping of Canopy Temp @ 14.31 NI





Summary
Trait: %14_31_NI

List of QTLs

Locus no.	Locus name	Linkage group	Position	log10(P)
495	BobWhite_c28333_454	5B	138.23	3.859

QTL effects

Locus no.	Locus name	%Expl. Var.	Add. eff.	High value allele	s.e.
495	BobWhite_c28333_454	9.171	0.277	Garcia	0.071

=Q5B-2

Wheat Genetic Improvement Network

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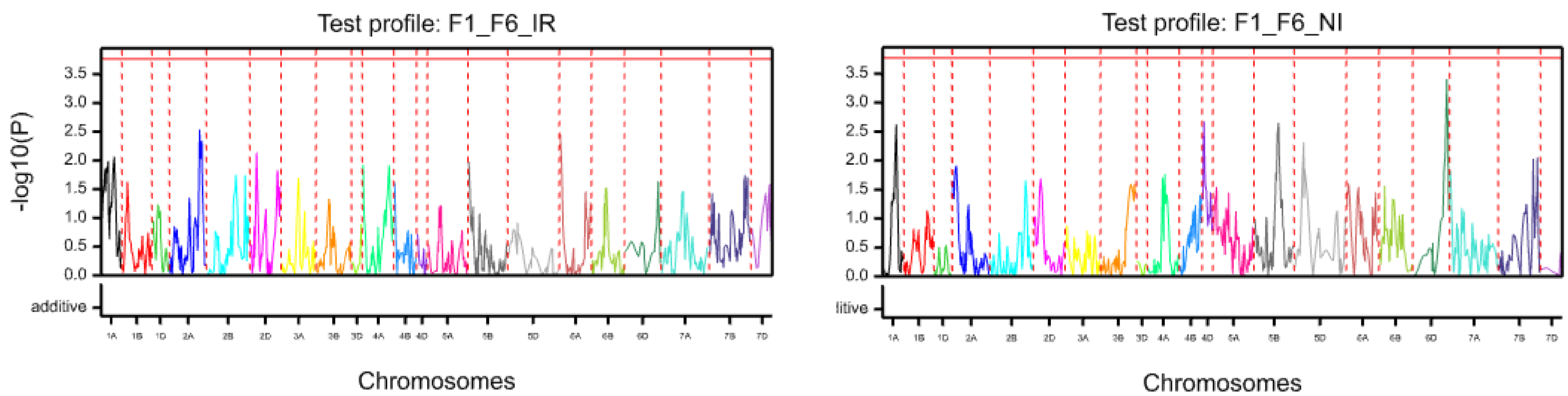
Department for Environment Food & Rural Affairs

ROTHAMSTED RESEARCH

QTL mapping of Canopy Temp 9.25 (F1)-14.31 (F6)



Wheat Genetic Improvement Network

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Department for Environment Food & Rural Affairs

ROTHAMSTED RESEARCH

Wheat Genetic Improvement Network

Summary of 2016-2018 Yield QTLs

John Innes Centre

2016 Spring Rain, little Summer Drought					2017 Severe spring Drought, some Summer Drought					2018 Spring Rain, severe Summer Drought					
	Chr	% expl var		QTL name		Chr	% expl var		QTL name		Chr	% expl var		QTL name	
TGWT NI	1A	18.2	Gar	Q1A-1	TGWT NI	1A	14.6	Gar	Q1A-1	TGWT NI	1A	13.3	Gar	Q1A-1	NIL production
TGWT IR	1A	15.0	Gar	Q1A-1						TGWT IR	1A	10.8	Gar	Q1A-1	NIL production
					YLD IR	1A	11.4	Gar	Q1A-2						
					SW IR	1A	5.0	Gar	Q1A-2						
					SW NI	2B	11.3	Gar	Q2B-1						Ppd-B1
					SW IR	2B	9.4	Gar	Q2B-1						Ppd-B1
					Grains / m2 IR	2B	16.2	Par	Q2B-1	Grains / m2 IR	2B	9.5	Par	Q2B-1	Ppd-B1
										Grains / m2 NI	2B	10.6	Par	Q2B-1	Ppd-B1
SW NI	2B	9.0	Gar	Q2B-2						SW NI	2B	6.1	Gar	Q2B-2	NIL production
					YLD NI	2B	17.7	Gar	Q2B-2	YLD NI	2B	16.6	Gar	Q2B-2	NIL production
					YLD IR	2B	17.0	Gar	Q2B-2	YLD IR	2B	13.9	Gar	Q2B-2	NIL production
Grains / m2 NI	2D	14.9	Gar	Q2D-1											
SW NI	3A	6.1	Par	Q3A-1											
TGWT NI	3B	4.5	Gar	Q3B-1											
SW NI	4D	29.2	Par	Q4D-1	SW NI	4D	33.7	Par	Q4D-1	SW NI	4D	28.5	Par	Q4D-1	*Rht*
SW IR	4D	38.1	Par	Q4D-1	SW IR	4D	33.0	Par	Q4D-1	SW IR	4D	37.1	Par	Q4D-1	*Rht*
					TGWT NI	4D	16.6	Par	Q4D-1						*Rht*
TGW IR	4D	5.2	Par	Q4D-1	TGW IR	4D	18.7	Par	Q4D-1	TGW IR	4D	11.1	Par	Q4D-1	*Rht*
					Grains / m2 NI	4D	25.4	Gar	Q4D-1	Grains / m2 NI	4D	12.7	Gar	Q4D-1	*Rht*
					Grains / m2 IR	4D	19.1	Gar	Q4D-1	Grains / m2 IR	4D	15.7	Gar	Q4D-1	*Rht*
Grains / m2 IR	5A	8.9	Gar	Q5A-1	Grains / m2 IR	5A	9.6	Gar	Q5A-1						
TGWT NI	5A	6.3	Gar	Q5A-2											
TGW IR	5A	6.5	Gar	Q5A-2											
SW IR	5A	6.8	Par	Q5A-2											
SW NI	5A	2.5	Gar	Q5A-3											
SW IR	5A	7.0	Gar	Q5A-3											
					TGWT NI	5A	5.0	Gar	Q5A-3						
					TGW IR	5A	7.1	Gar	Q5A-3						
					TGWT NI	5B	8.4	Gar	Q5B-1	TGWT NI	5B	10.7	Gar	Q5B-1	QTL of interest
TGW IR	5B	14.2	Gar	Q5B-1	TGW IR	5B	9.2	Gar	Q5B-1	TGW IR	5B	14.6	Gar	Q5B-1	
					Grains / m2 NI	5B	7.1	Par	Q5B-2						
										YLD IR	6A	8.9	Gar	Q6A-1	QTL of interest
					TGWT NI	7A	12.3	Gar	Q7A-1	TGWT NI	7A	7.7	Gar	Q7A-1	
					TGW IR	7A	8.2	Gar	Q7A-1						
Grains / m2 NI	7A	12.0	Par	Q7A-1	Grains / m2 NI	7A	7.8	Par	Q7A-1	Grains / m2 NI	7A	10.7	Par	Q7A-1	
YLD NI	7B	16.8	Gar	Q7B-1											
					SW NI	7D	5.2	Gar	Q7D-1						

KEY
Garcia increasing allele
Paragon increasing allele
IR = Irrigated plots
NI = Not-irrigated plots
YLD = Yield
SW = Specific Wt
TGWT = 1000 Grain Wt
Grains / m2 = Yield/TGWT
% expl var >10%

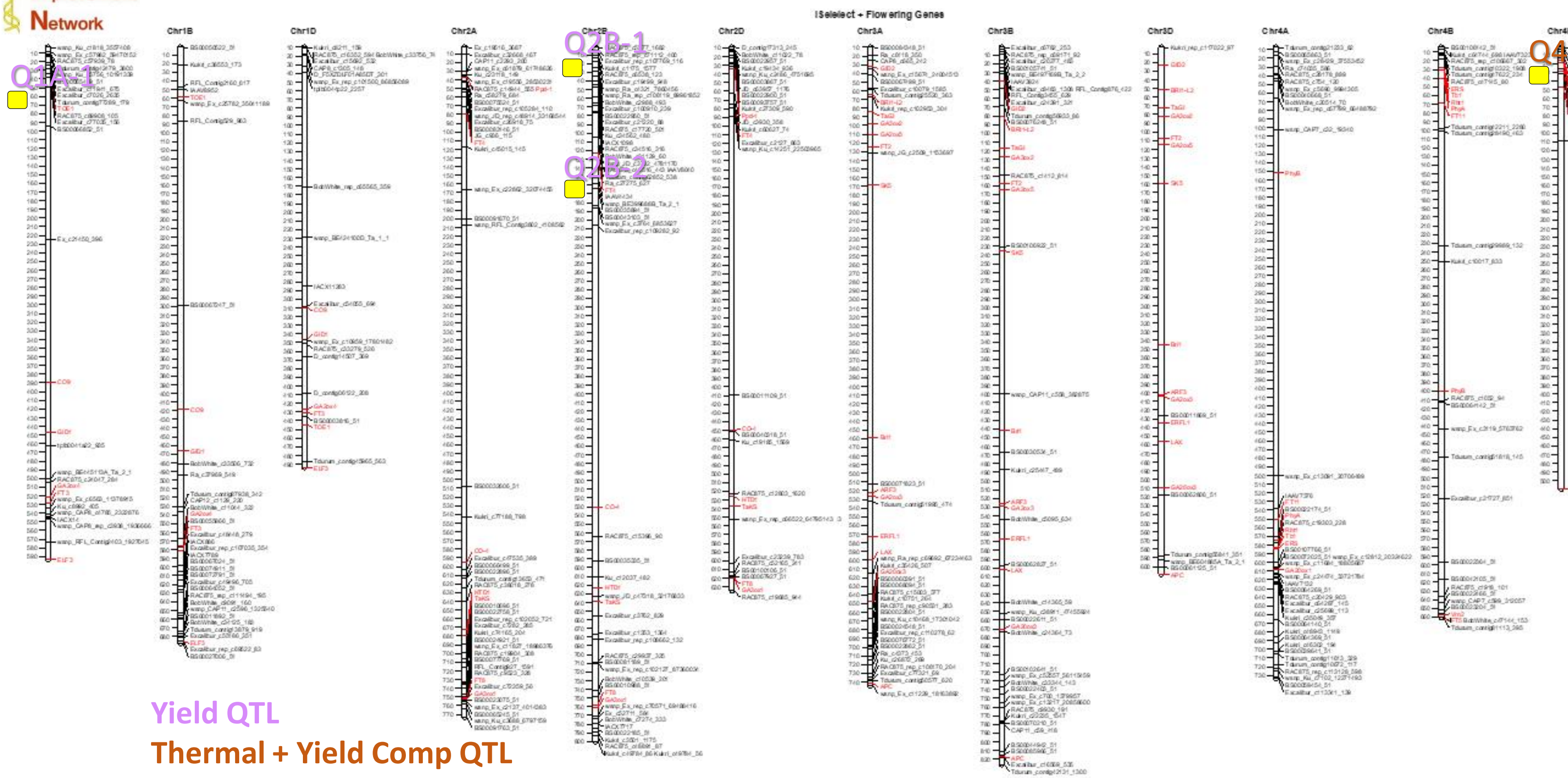
Department for Environment Food & Rural Affairs

ROTHAMSTED RESEARCH

Wheat Genetic Improvement Network

iSelect map + Flowering Genes + QTLs

John Innes Centre



Yield QTL

Thermal + Yield Comp QTL

Thermal QTL

Thermal + Flowering QTL

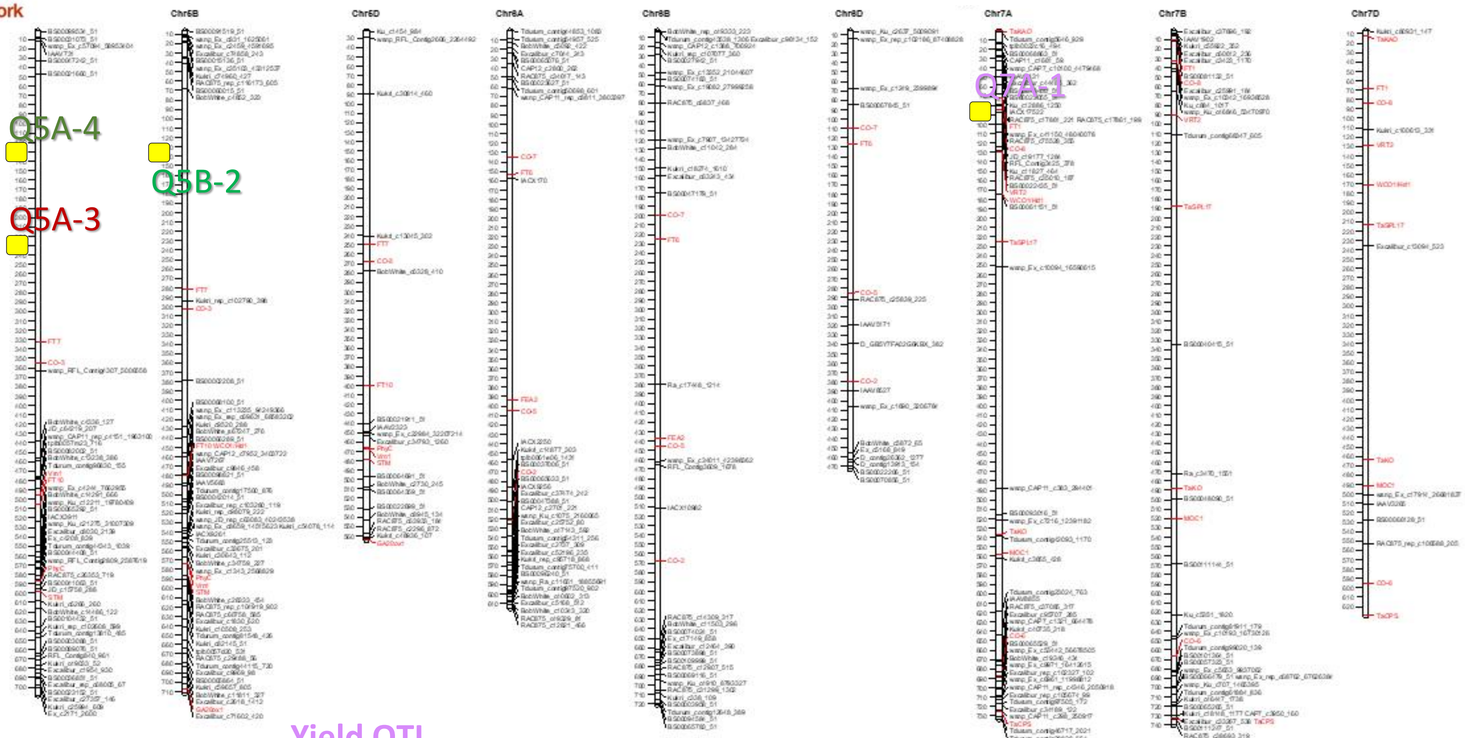
Department for Environment Food & Rural Affairs

ROTHAMSTED RESEARCH

Wheat Genetic Improvement Network

iSelect map + Flowering Genes + QTLs

John Innes Centre



Yield QTL

Thermal + Yield Comp QTL

Thermal QTL

Thermal + Flowering QTL

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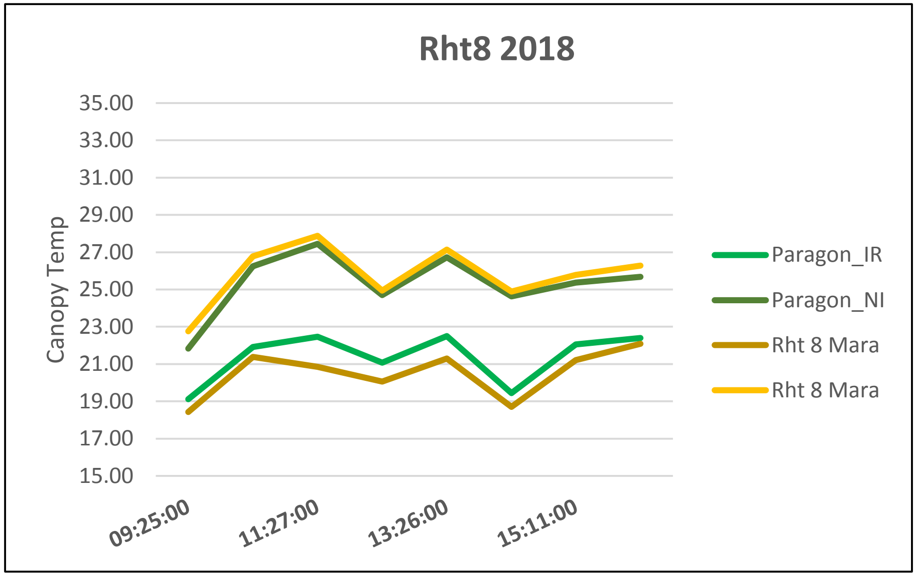
ROTHAMSTED RESEARCH

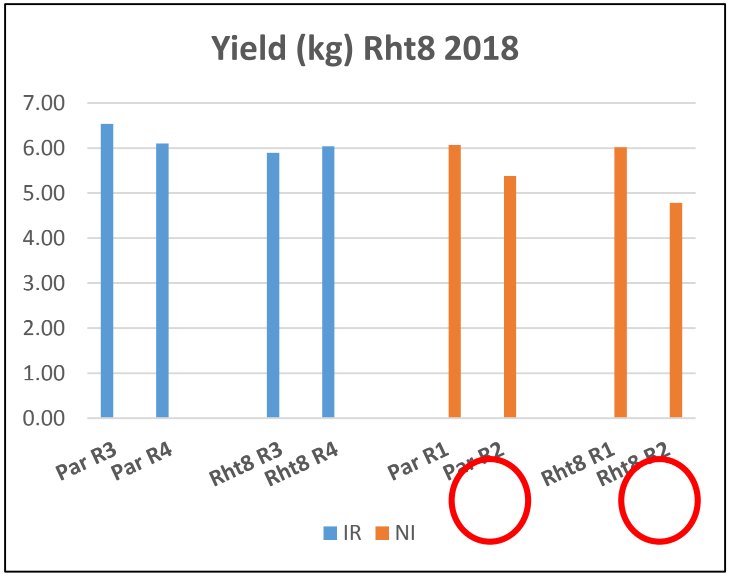
Subset of Paragon Library in Drought Trials

Paragon	
Lr19 Kamb1	Alien introgression*
Par Mutant 2316b	Staygreen
Ppd 1x Early	DTEM
Ppd 2x Early	DTEM
Ppd 3x Early	DTEM
Ppd KO 2x	DTEM
Rht 8 Mara	Height
Rht B1 Robigus	Height
Rht D1 Alchemy	Height

*Leaf rust resistance gene on 7DL, derived from *Agropyron elongatum*

Canopy Temp and Yield of Paragon Library





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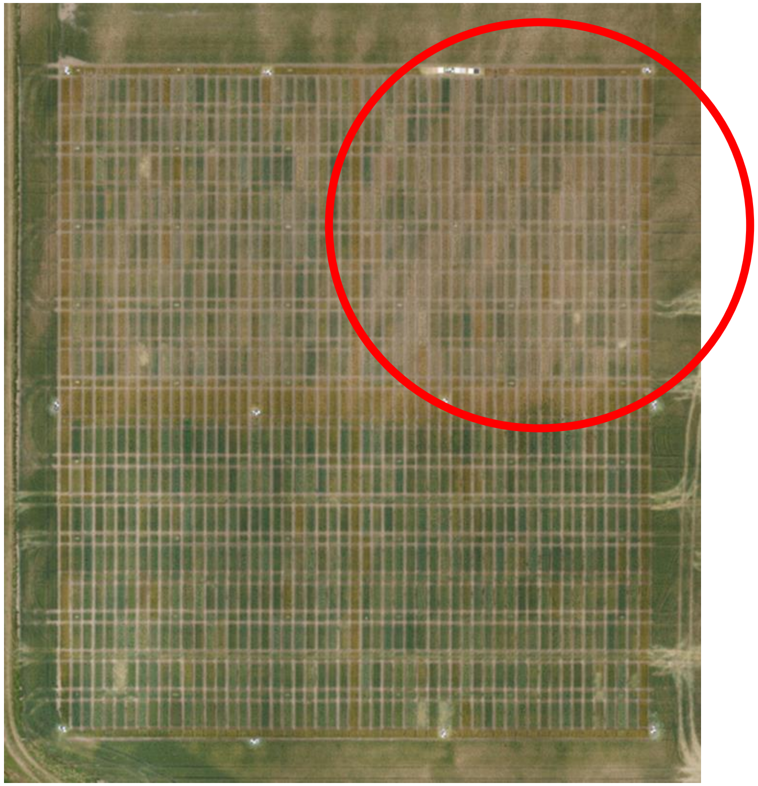
ROTHAMSTED RESEARCH

Canopy Temp and Yield of Paragon Library



Rep 2 is particularly poor in 2018!

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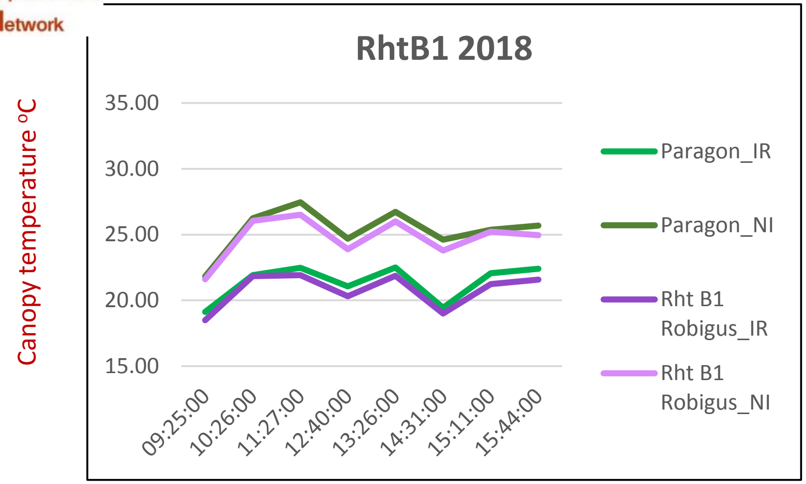
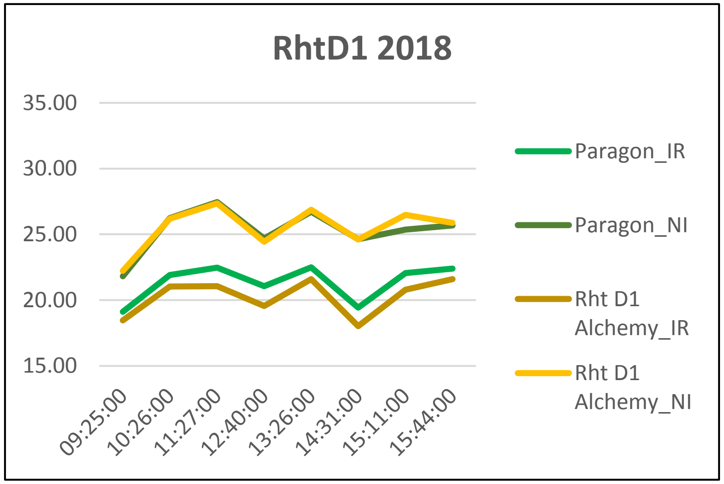
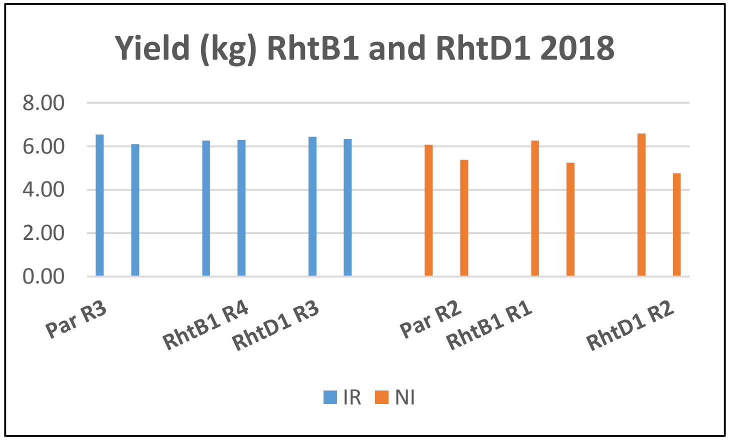
ROTHAMSTED RESEARCH

Canopy Temp and Yield of Paragon Library



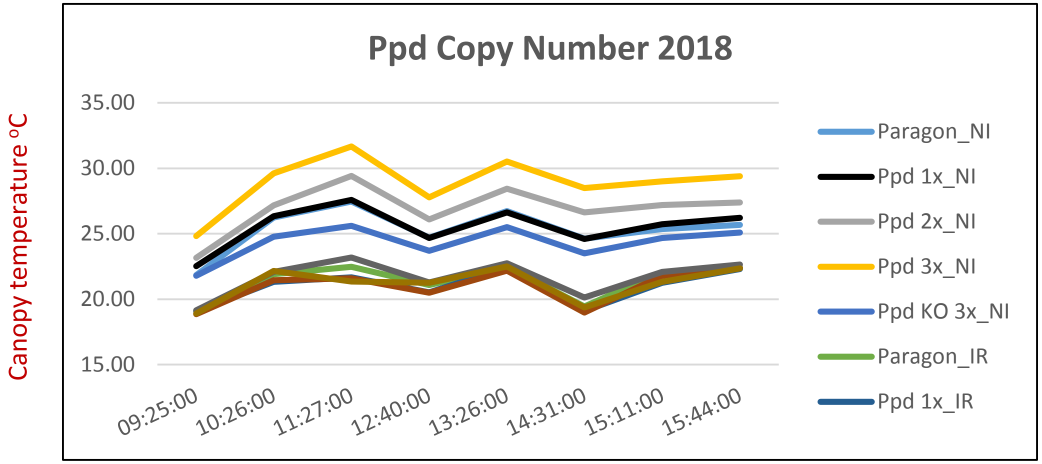


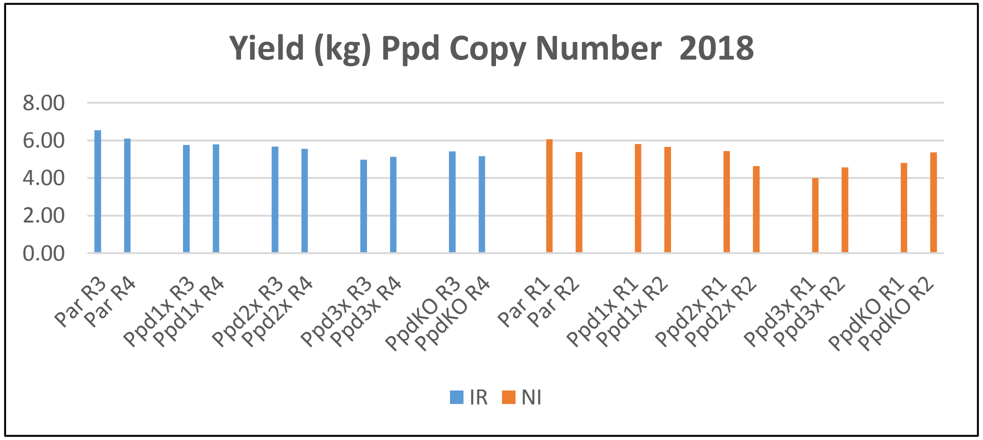


Department for Environment Food & Rural Affairs

ROTHAMSTED RESEARCH

Canopy Temp and Yield of Paragon Library





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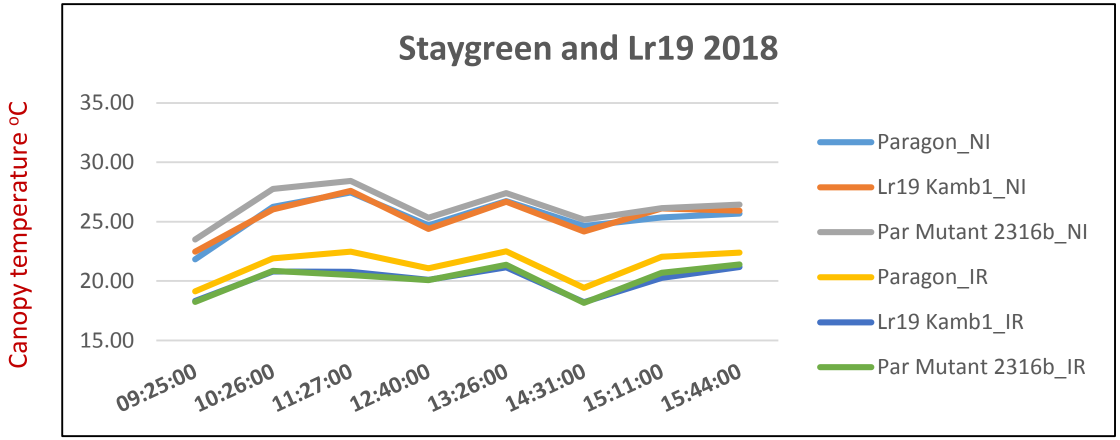
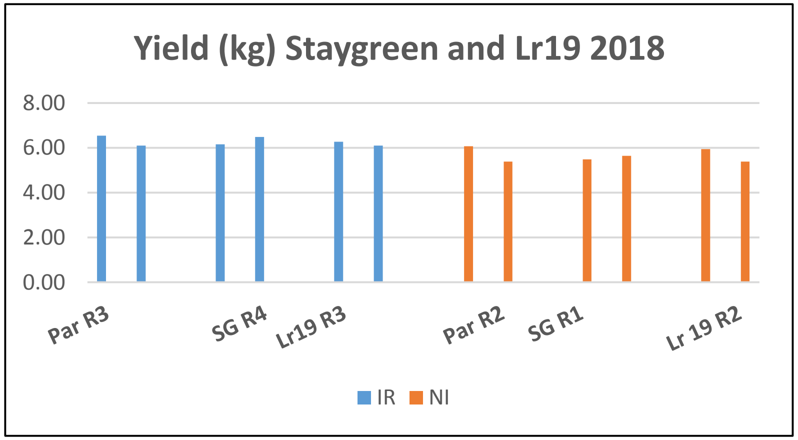
ROTHAMSTED RESEARCH

Canopy Temp and Yield of Paragon Library





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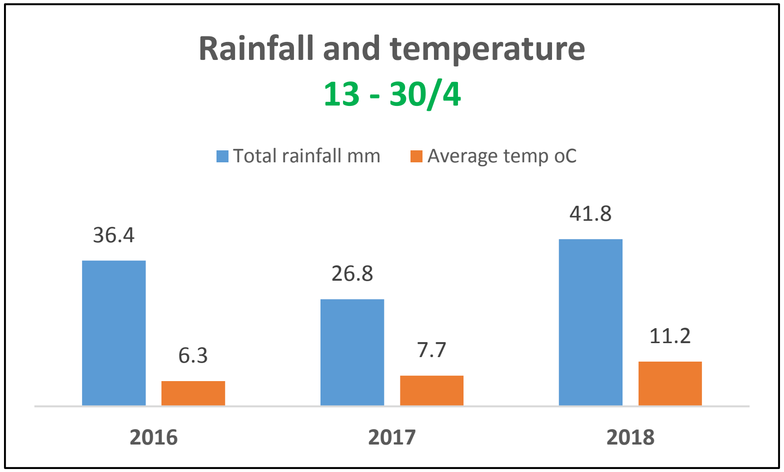
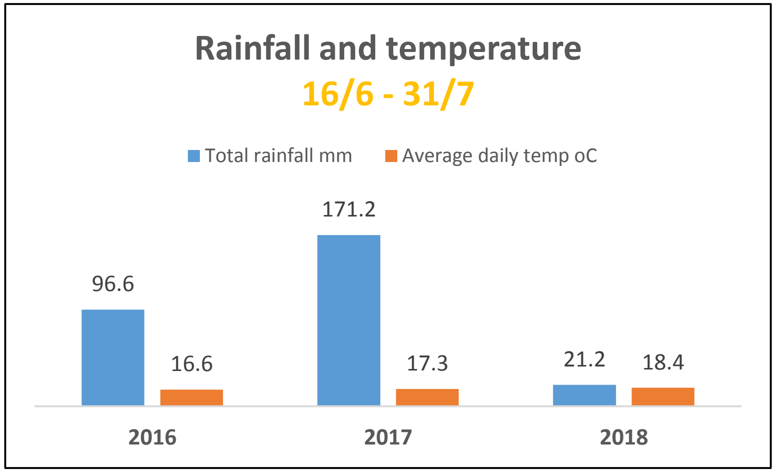
We are not just looking at Spring Drought!





Drought trial drilled again in October 2018….

ROTHAMSTED RESEARCH

BYDV and wheat

Dr Gia Aradottir

Cereal aphids & Barley Yellow Dwarf Virus

ROTHAMSTED RESEARCH

Bird cherry-oat aphid

English grain aphid

BYDV epidemic Barrow, Suffolk 2016
(© Dewar Crop Protection Ltd)

WGIN Diversity trial - field

ROTHAMSTED RESEARCH

1	Avalon
2	Barrel
3	Cadenza
4	Claire
5	Crusoe
6	Graham
7	Hereward
8	Hylux
9	Istabraq
10	Malacca
11	Maris Widgeon
12	Mercia
13	Paragon
14	Robigus
15	Riband
16	Siskin
17	Soissons
18	Solstice
19	Xi19
20	Zyatt

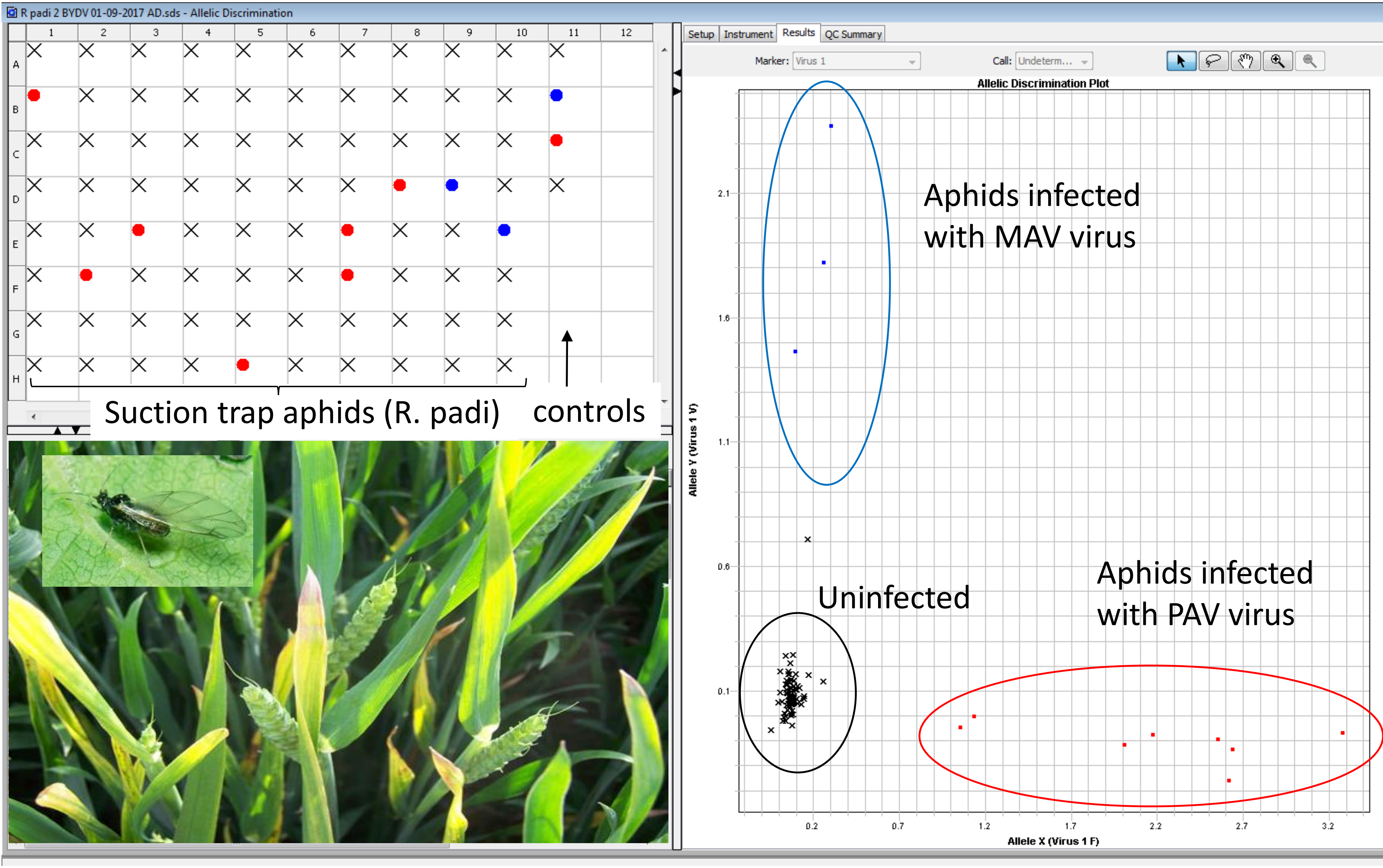
WGIN Diversity trial - glasshouse

ROTHAMSTED RESEARCH

- Replicate of lines in the field
- Infected with BYDV-PAV
- 1st visual and Taqman assay 1 week after infection
- Plants in the vernaliser
- Scoring
    - Visual symptoms
    - Molecular markers
    - Agronomic traits

A real-time PCR assay for detecting BYDV in cereal aphids

ROTHAMSTED RESEARCH



Colony virulence

ROTHAMSTED RESEARCH

- Ability of different aphid colonies to take up and transmit BYDV
- Need MAV in culture & more colonies

Ramiro Morales- Hojas
Molecular Ecologist

Next steps

ROTHAMSTED RESEARCH

- Next 20 lines (selected from aphid phenotyping)
- Others if nominated
- Sample diversity trial for taqman assay
- Visit farmers for BYDV sampling (AHDB)

Rothamsted Research
where knowledge grows

Resilience to foliar and root fungal pathogens

Vanessa McMillan

Wheat Genetic Improvement Network

Department for Environment Food & Rural Affairs

WGIN MM 14th February 2019

BBSRC bioscience for the future

ROTHAMSTED RESEARCH

WGIN 4 objectives

ROTHAMSTED RESEARCH

- Resistance to septoria leaf blotch
- Resistance to yellow rust
- 3N ancestral introgression rooting trait
- Resistance to take-all disease in *Triticum monococcum*
- ***mlo* mediated resistance to powdery mildew**

mlo mediated resistance to powdery mildew

- Discovered in Barley in 1930s/1940s
- Loss-of-function mutations in Mildew resistance locus (*Mlo*) gene confers recessively inherited resistance against powdery mildew
- Non-race specific/broad spectrum
- Widely used in spring barley breeding programmes since 1970s/1980s
- Durable resistance; effective for > 30 years

Pleiotropic effects

ROTHAMSTED RESEARCH

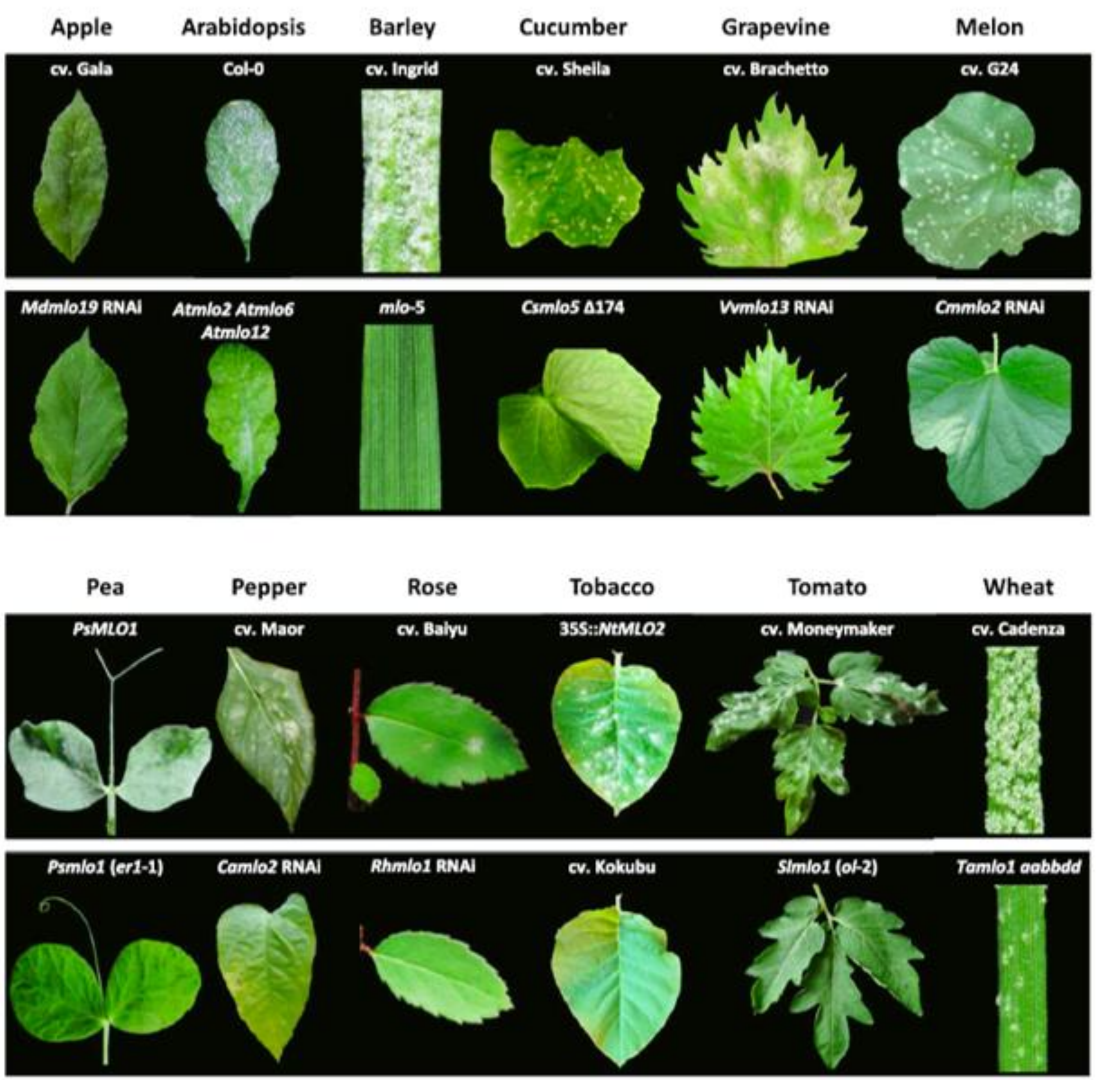
- Increased resistance to the hemibiotrophic oomycete *Phytophthora palmivora* (in young leaf sections only)
  Le Fevre *et al*. 2016. MPMI

- Increased susceptibility to rice blast fungus *Magnaporthe grisea, Fusarium graminearum* and *Ramularia collo-cygni* under glasshouse conditions
  Jarosch *et al*. 1999. MPMI. Jansen *et al*. 2005. PNAS. McGrann *et al*. 2014. J Exp Bot.

- Early leaf senescence > leaf chlorosis and necrosis > seen in both glasshouse and field conditions > impact on yield?
  Wolter *et al*. 1993. Mol Gen Genet. Makepeace *et al*. 2007. Plant Pathol.

BBSRC bioscience for the future

mlo based resistance is effective in many plant species



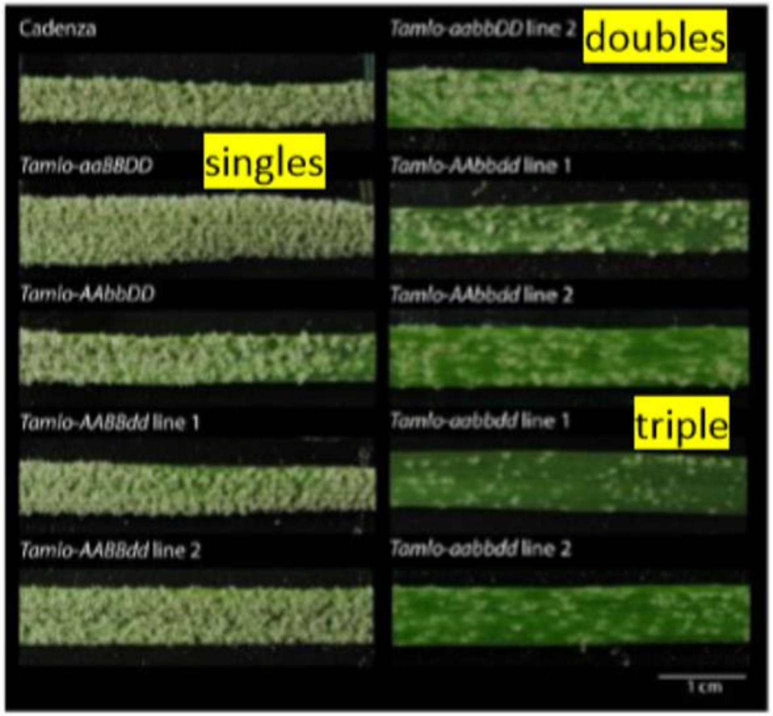
Kusch and Panstruga (2017) MPMI

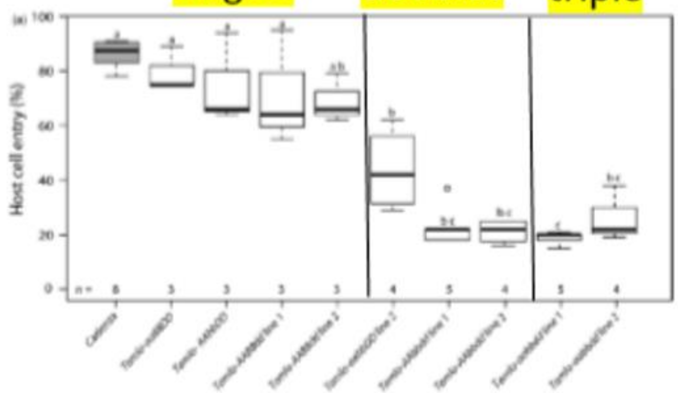
ROTHAMSTED RESEARCH

BBSRC bioscience for the future

TILLING wheat for mlo mediated mildew resistance

ROTHAMSTED RESEARCH





Cv Cadenza

Initially WGIN funded

Seedling glasshouse screen

Plant Biotechnology Journal 2017

Plant Biotechnology Journal (2017) 15, pp. 367–378

doi: 10.1111/pbi.12631

***mlo*-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach**

Johanna Acevedo-Garcia[1], David Spencer[1], Hannah Thieron[1], Anja Reinstädler[1], Kim Hammond-Kosack[2], Andrew L. Phillips[2] and Ralph Panstruga[1]*

Funders: German Federal Ministry of Food and Agriculture

Germany Society for the Advancement of Plant Innovation

BBSRC bioscience for the future

Are there trade offs under field conditions?

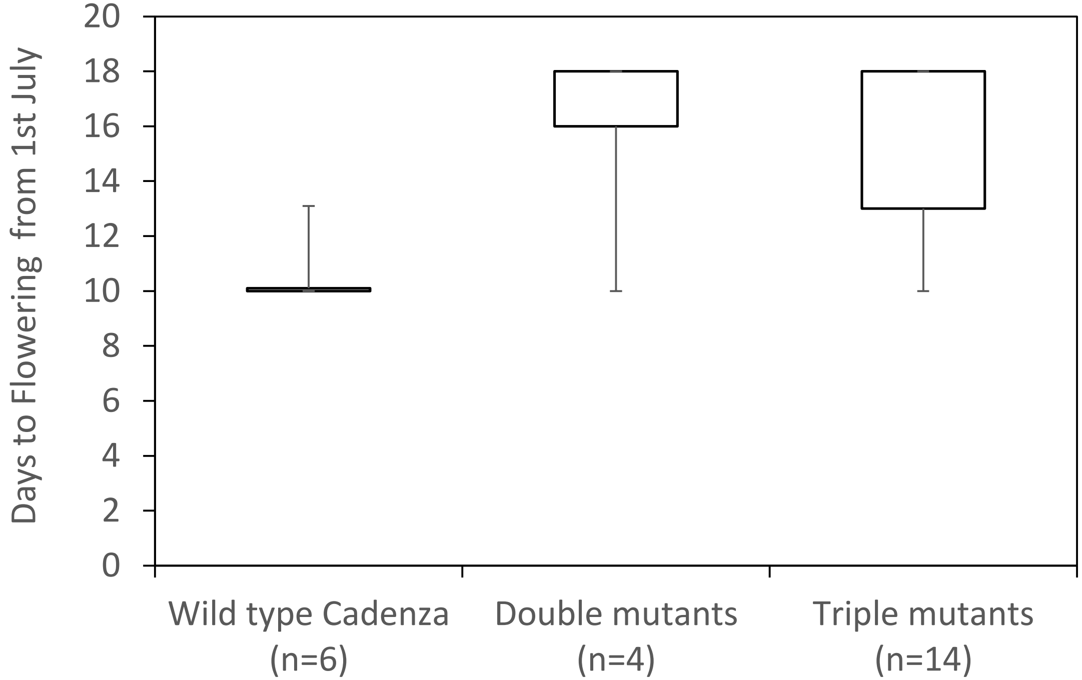
ROTHAMSTED RESEARCH

2017/18 Spring sown multiplication field trial

- 18 selected double and triple mutants (single replicate, 30 seeds)
- Replicated plots of Cadenza wildtype
- Foliar disease observations
- Ear emergence and plant heights

Days to Flowering

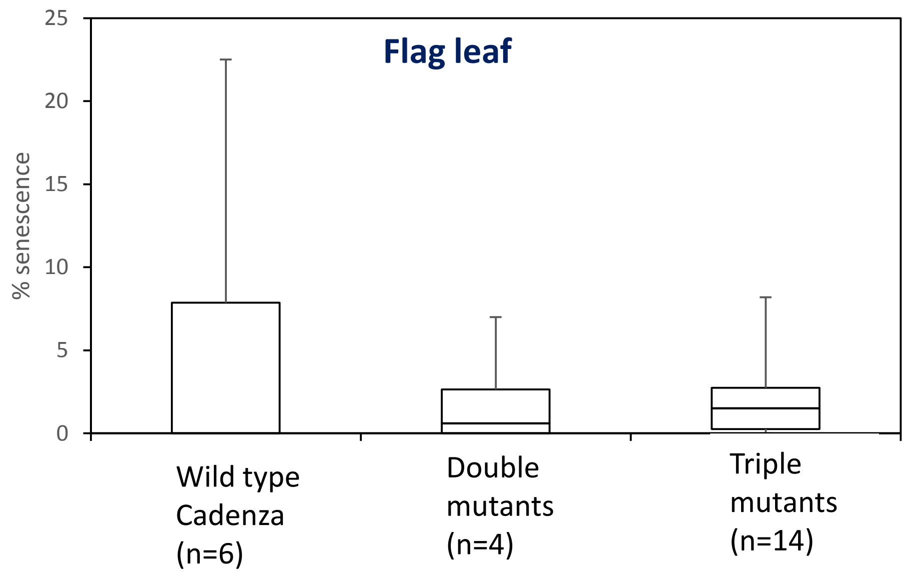
6 Cadenza plots, 4 double mutants, 14 triple mutants

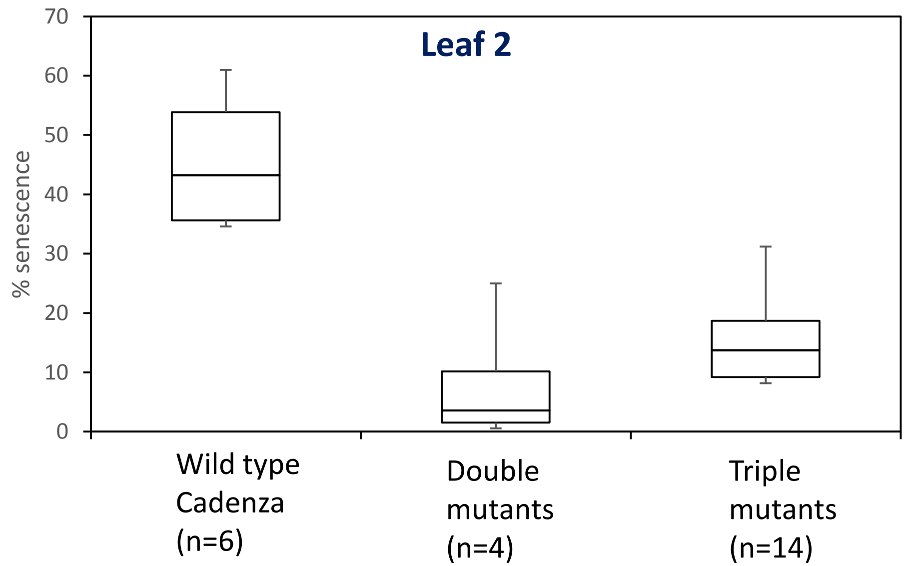


Many genotypes started flowering before full ear emergence due to drought conditions

Cadenza = earlier flowering that double or triple mutants

Senescence



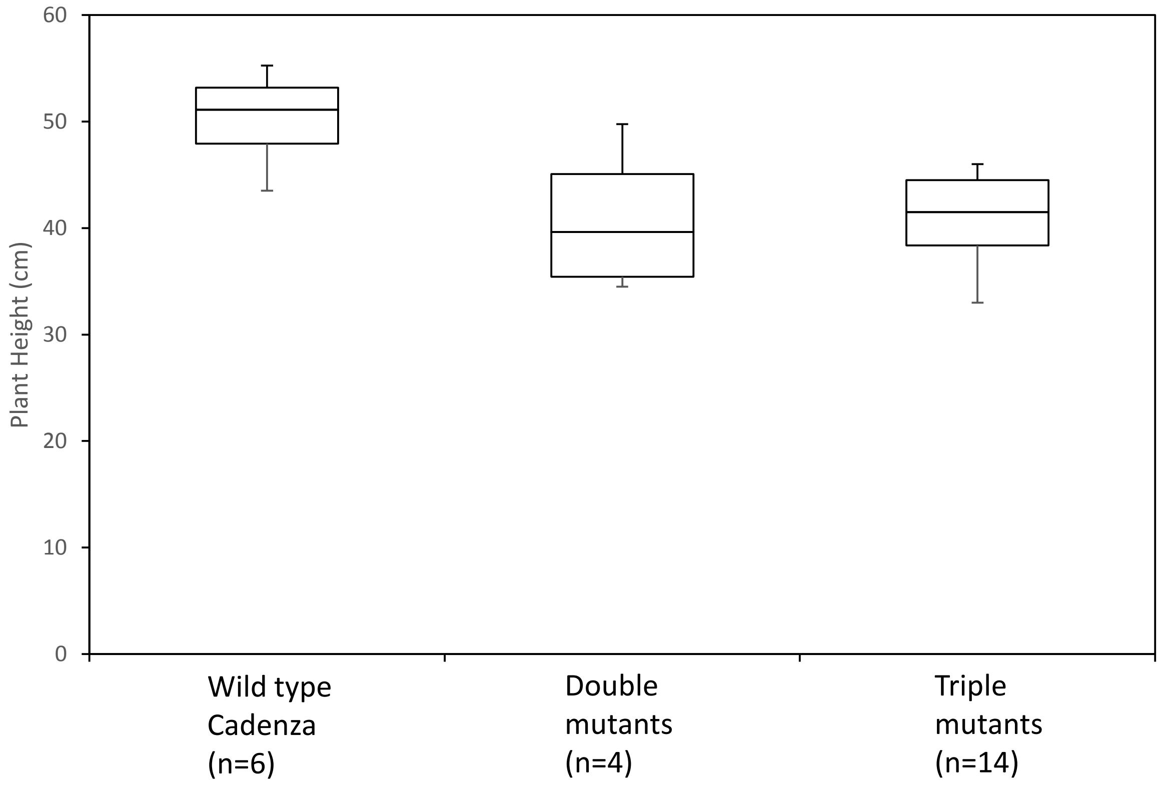


Cadenza plots much more senesced…related to earlier ear emergence?

Impact of drought?

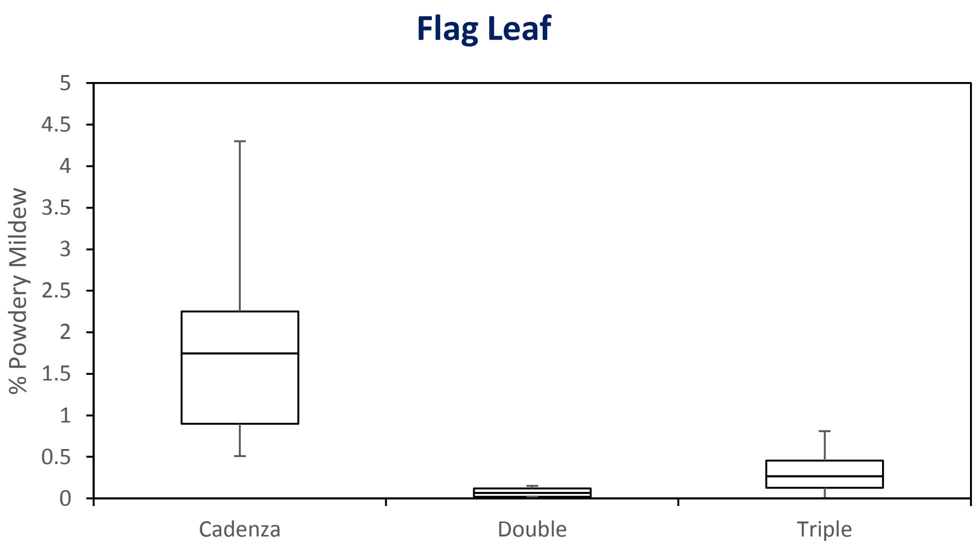
Plant Height

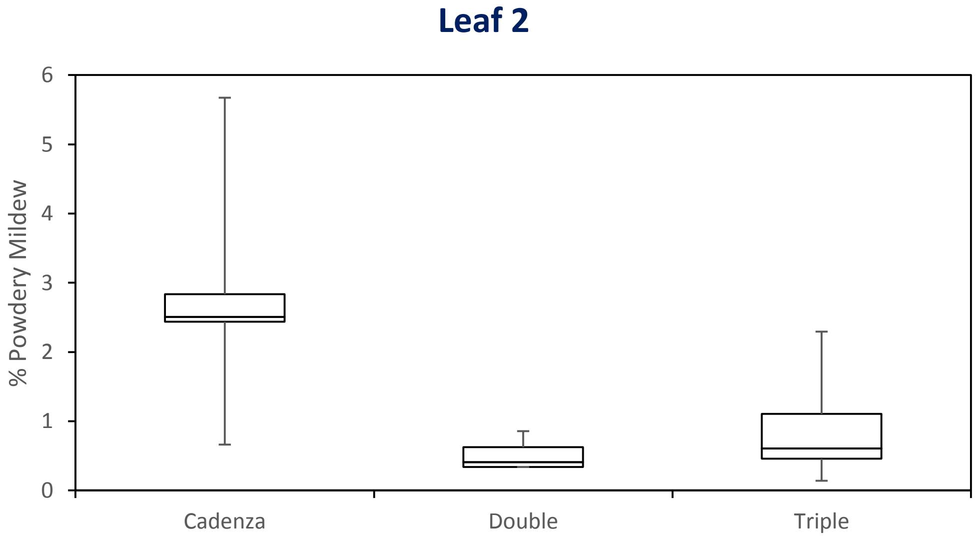
6 Cadenza plots, 4 double mutants, 14 triple mutants



Wildtype Cadenza slightly taller?

Powdery Mildew



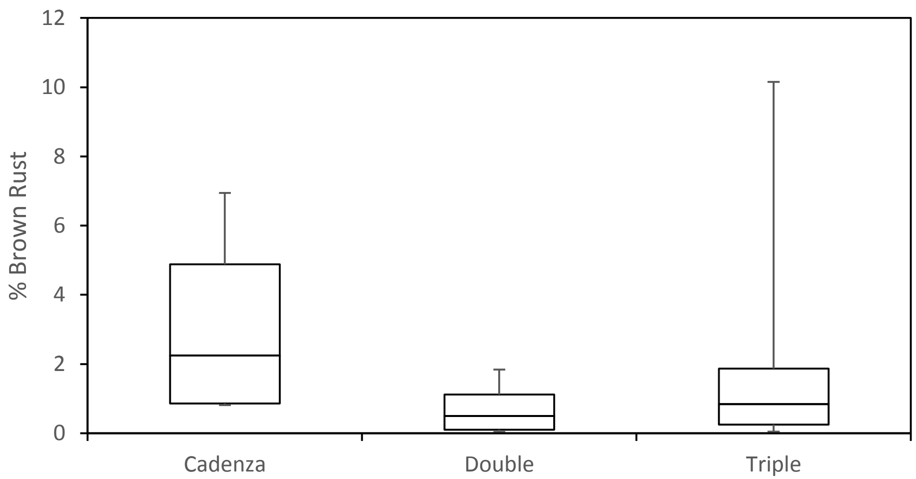


Low powdery mildew disease pressure

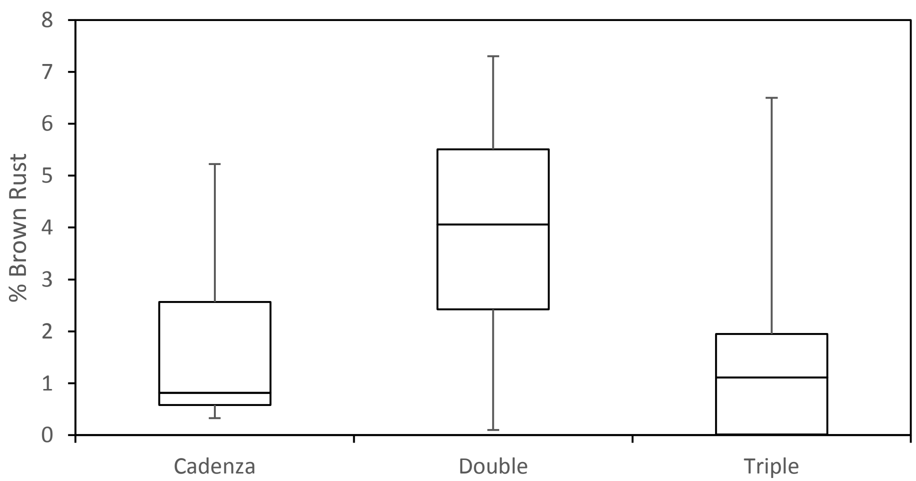
Trend for a reduction in powdery mildew infection for double and triple mutants

Brown Rust

Flag leaf



Leaf 2



Overall relatively low brown rust disease levels across field trial

A couple of triple mutants with relatively high brown rust scores – perhaps due to location in trial?

ROTHAMSTED RESEARCH

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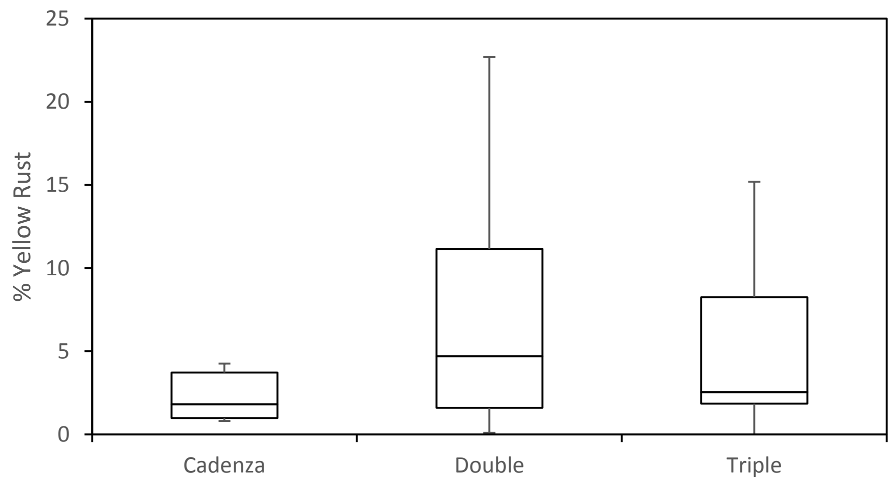
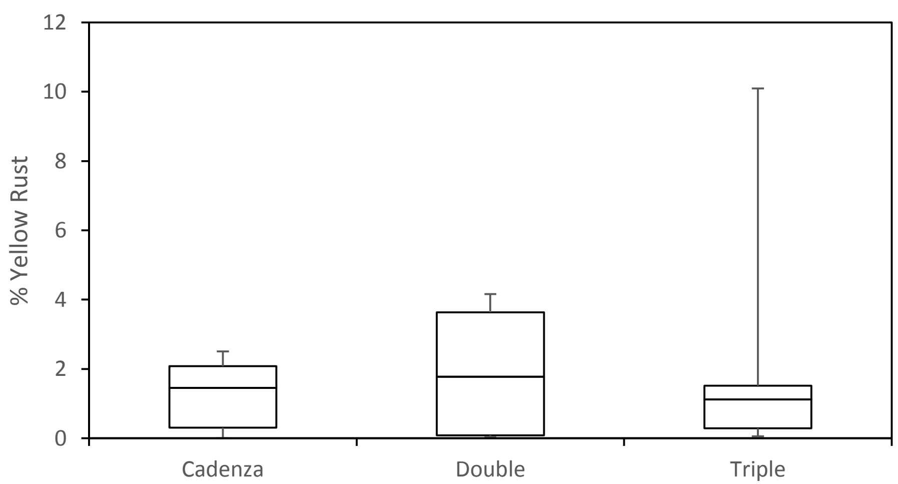
Yellow Rust

Flag Leaf



Leaf 2



Some doubles and triples with higher yellow rust infection

mlo mediated resistance in wheat

ROTHAMSTED RESEARCH

2019 and 2020

Replicated field trials (4 replicates per mutant line) to explore the double and triple lines susceptibility/resistance to additional pathogens (as a winter crop) – yellow rust, brown rust, septoria and fusarium

Leaf senescence and other morphological differences to be assessed

Trials drilled 23rd October 2018

BBSRC bioscience for the future

Delayed plant emergence

18th January 2019

Most lines have at least 2 leaves fully emerged
Some plots with only one leaf half emerged
No sign of rabbit or bird damage

Delayed plant emergence

Mutants with slow plant emergence

Poorly emerged treatments	MLO genotype	Line	*Tamlo-A1*	*Tamlo-B1*	*Tamlo-D1*
34.2-33 (8 replicates)	aabbdd	1	P324L	G318R	P334L
36.2-30 (4 replicates)	aabbdd	1	P324L	G318R	P334L
40.3-30 (6 replicates)	AAbbdd	2	WT	G318R	G318R
52.3-82 (8 replicates)	aabbdd	4	P324L	T296I	P320S

- Phenotype very consistent across replicates and field sites
- Does not seem to be related to mutant allele combinations
- Effect of background mutations?

Many thanks to

ROTHAMSTED RESEARCH

Kim Hammond-Kosack
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Mike Hammond-Kosack
Gail Canning
Carlos Bayon
Tania Chancellor (2nd year PhD student)
Jessica Hammond (Plant Pathology Apprentice)

Summer students
Erin Baggs (2015)
Eleanor Leane (2015)
Tessa Reid (2015)
Laurie Neal (2015,2016&2017)
Alex Chambers-Ostler (2016)
Leanne Freeman (2016,2017&2018)
Jamie Hawkesford (2017)
Ellen Farnham (2017&2018)
Georgie Halford (2018)
Eoin Canning (2018)
Niamh Kavanagh (2018)

Rodger White and Stephen Powers - statistics

RRes Farm and glasshouse staff

Wheat Genetic Improvement Network

Department for Environment Food & Rural Affairs

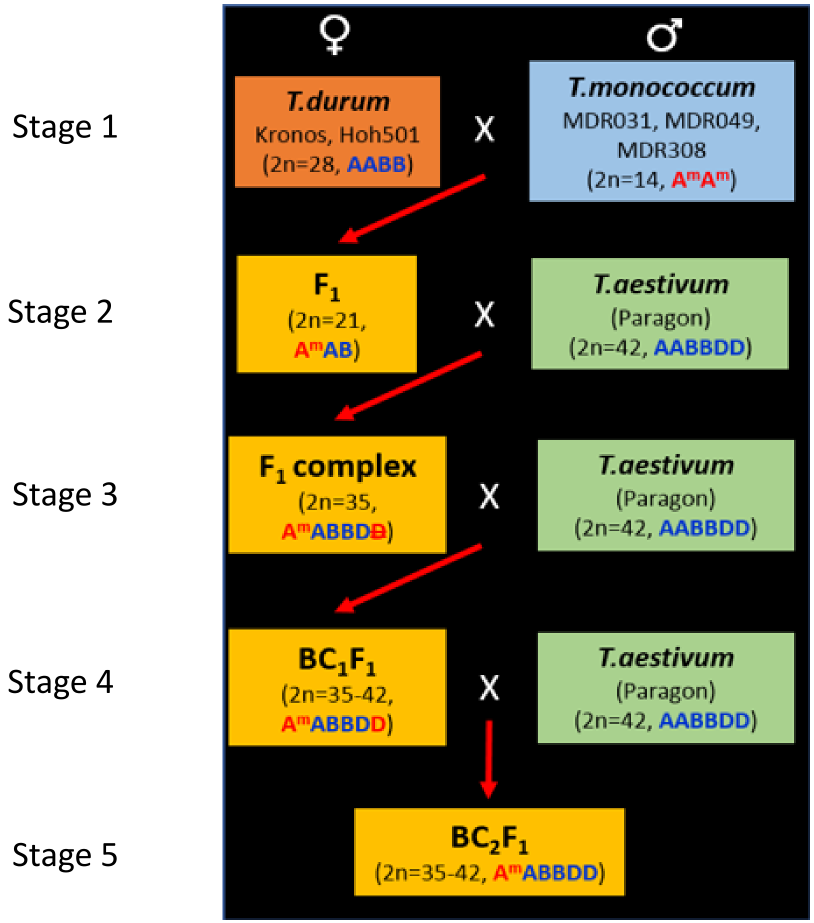
BBSRC bioscience for the future

Triticum monococcum Introgression

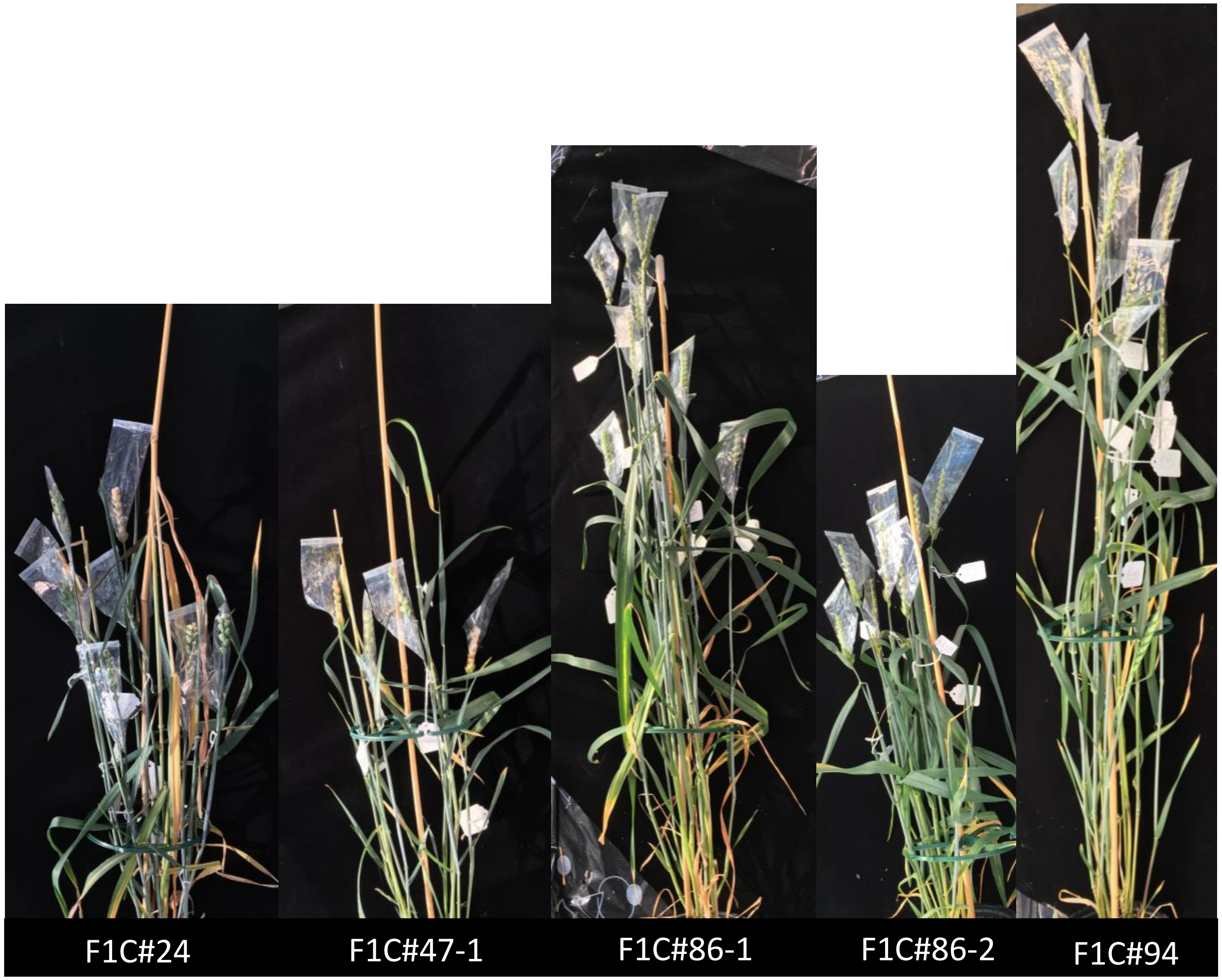
WGIN MM February 14th 2019

Michael Hammond-Kosack

Crossing Strategy – Tetraploid Wheat as a Bridging Species



Fertile F_1 Complex Plants generated in stage 2 and pollinated in stage 3



Comparison of Hoh501 vs Kronos as mother

1. Grain setting

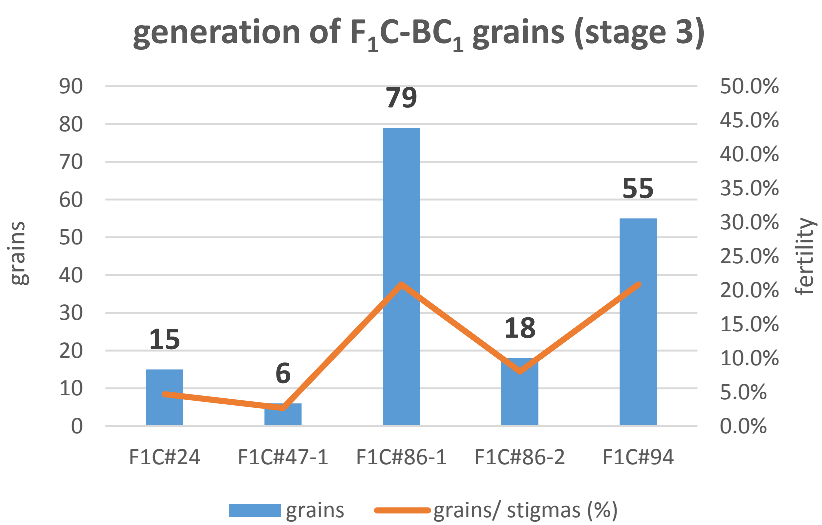
		Kronos		Hoh501		Hoh501/Kronos	
		grains	fertility	grains	fertility	grains (n-fold difference)	fertility (n-fold difference)
stage 1	Tdur x Tm = F1_hybrids	35	3.37%	100	8.31%	2.9	2.5
stage 2	F1_hybrids x Taes = F1C	9	0.46%	25	0.27%	2.8	0.6
stage 3	F1C x Taes = F1C_BC1	21	3.45%	152	16.96%	7.2	4.9

2. pre-germination

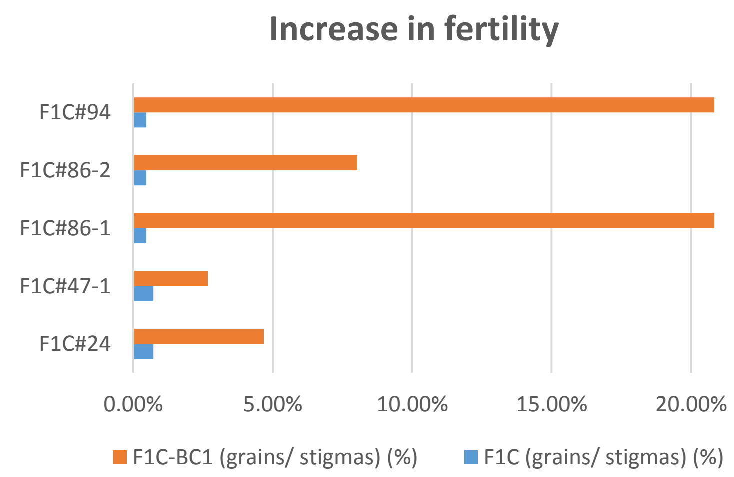
Hoh501 30% (28/94) after 5 days
Kronos 60% (13/22) after 4 days

Phenotypes Observed in F_1 Hybrid and F_1 Complex Plants

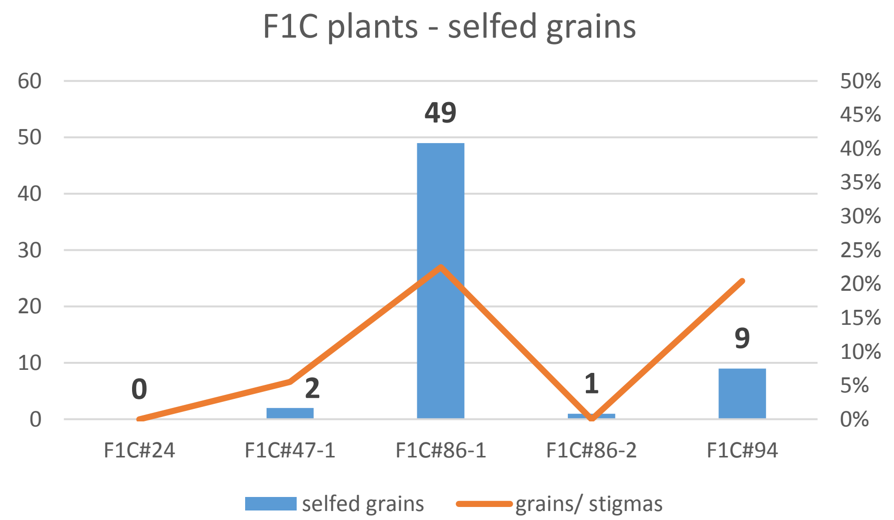
additional leaf: both on F1-hybrid (L) & F1C plants (R) | extending spikelet | Disrupted ear architecture



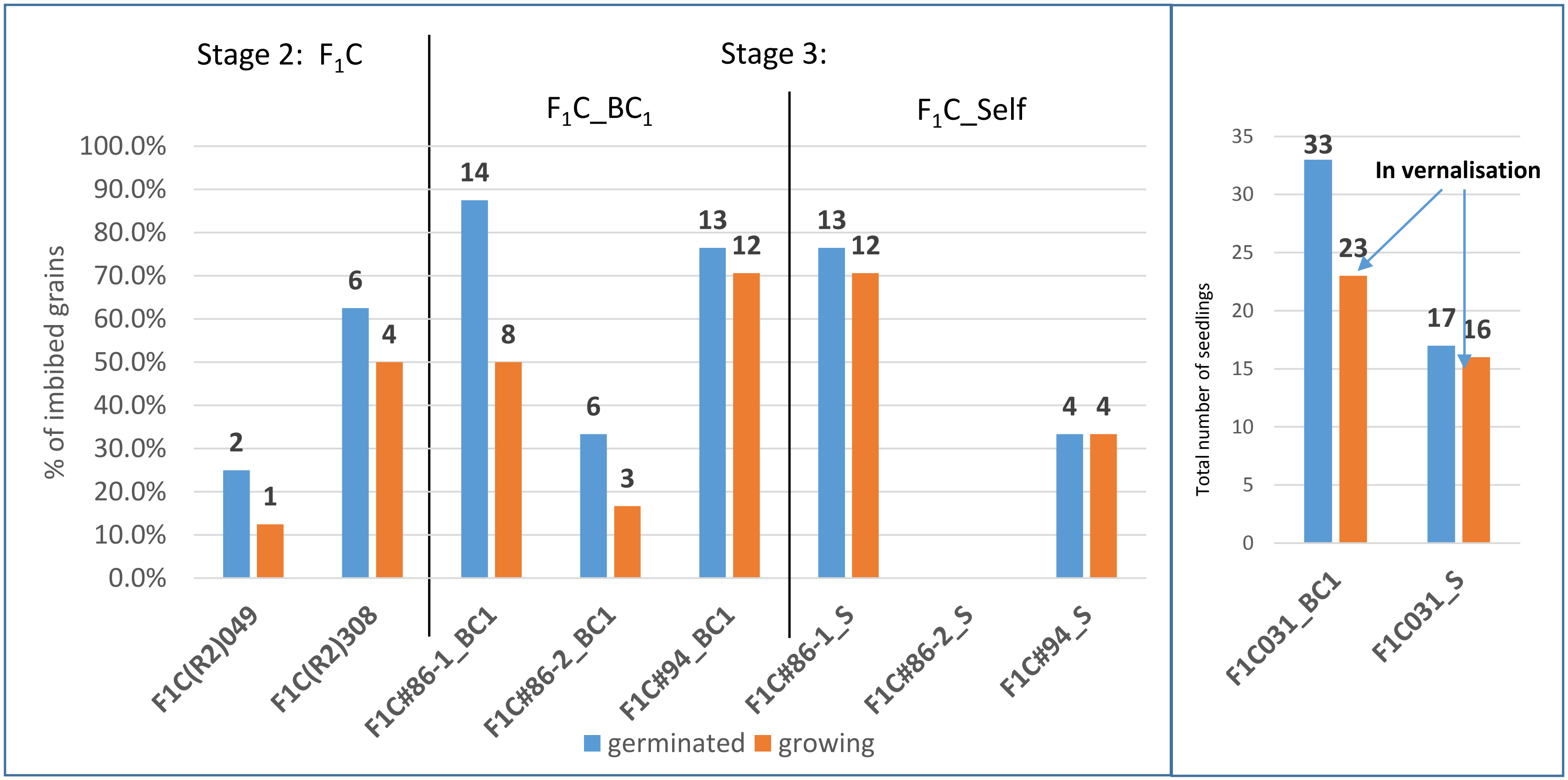
generation of $F_1C\text{-}BC_1$ grains (stage 3)
90
80
70
60
50
40
30
20
10
0
grains
79
55
15
6
18
50.0%
45.0%
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
fertility
F1C#24
F1C#47-1
F1C#86-1
F1C#86-2
F1C#94
grains
grains/ stigmas (%)



Increase in fertility
F1C#94
F1C#86-2
F1C#86-1
F1C#47-1
F1C#24
0.00%
5.00%
10.00%
15.00%
20.00%
F1C-BC1 (grains/ stigmas) (%)
F1C (grains/ stigmas) (%)



F1C plants - selfed grains
60
50
40
30
20
10
0
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
49
0
2
1
9
F1C#24
F1C#47-1
F1C#86-1
F1C#86-2
F1C#94
selfed grains
grains/ stigmas



Hoh501 as mother

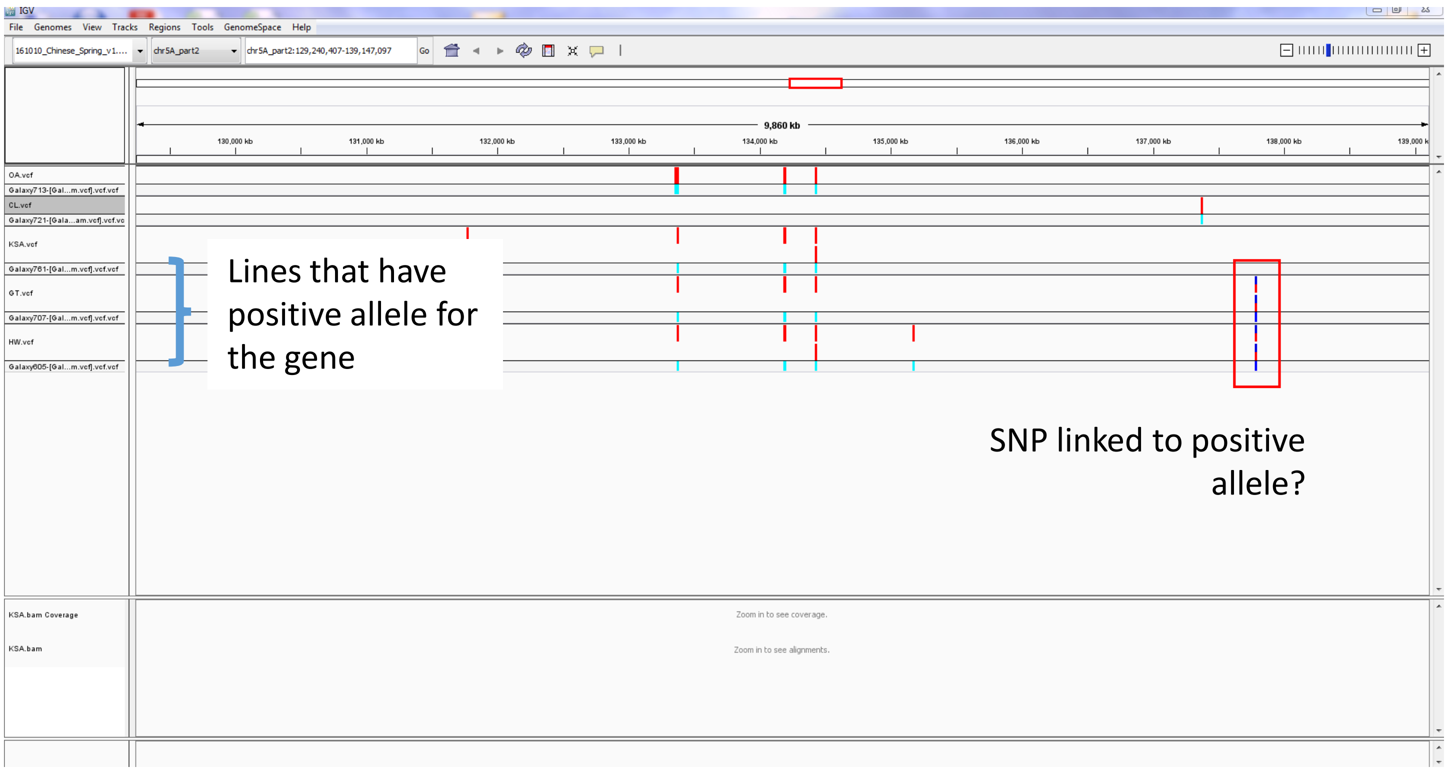
WGIN Promotome Capture

WGIN MM February 19th 2019

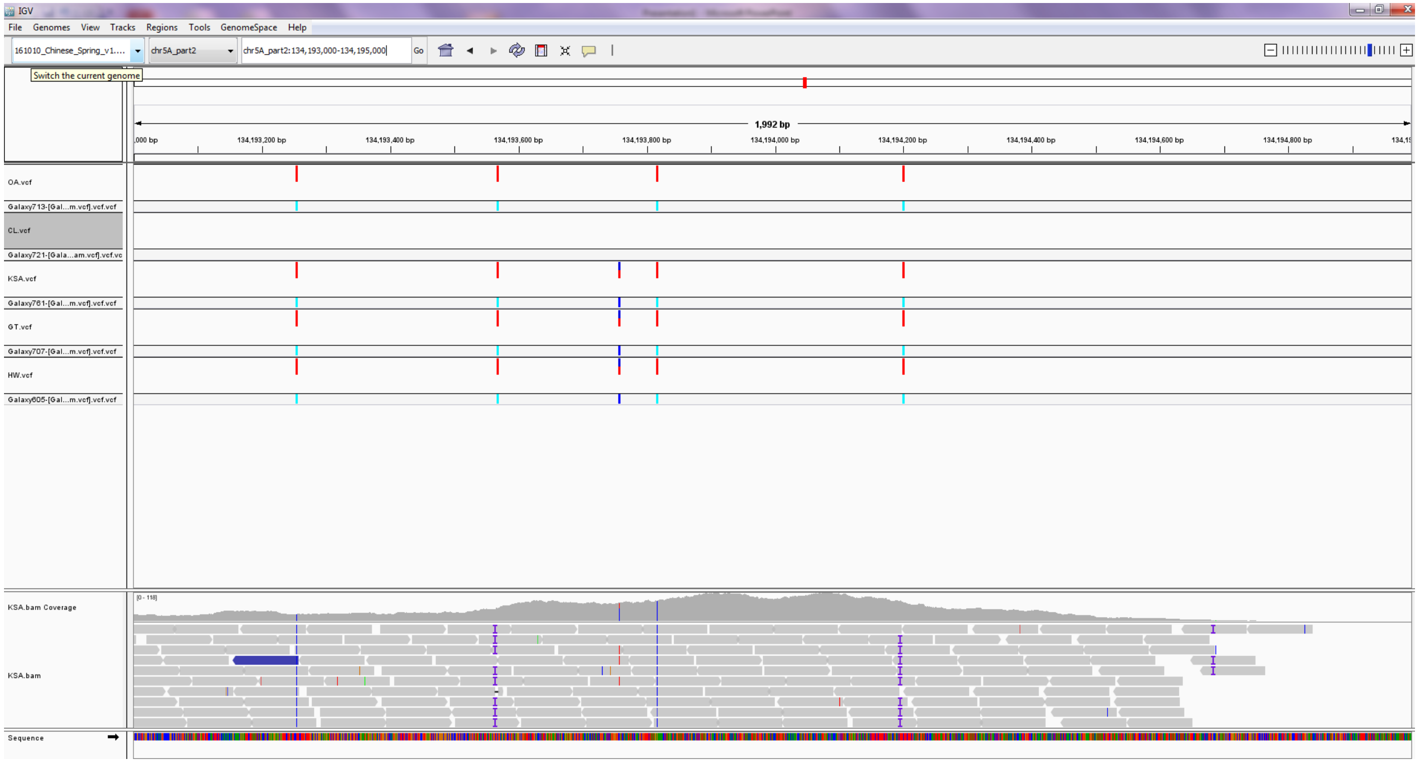
Slides below provided by Chris Burt, RAGT

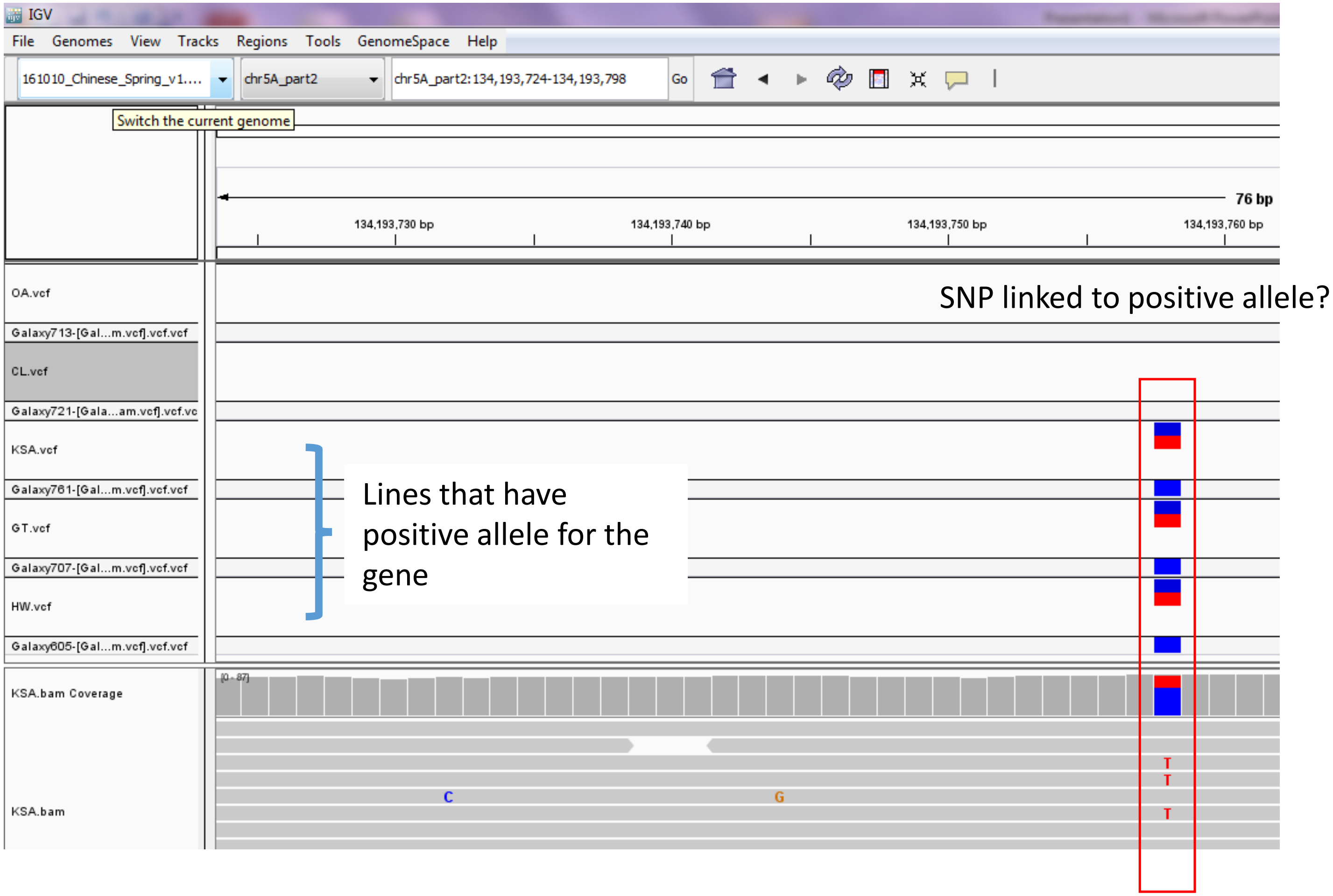
Import .vcf and .BAM files into IGV for varieties with different alleles of targeted major gene

~1Mb region including targeted major gene



Zoom into SNPs in the region to see read information in BAM tracks.





IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
161010_Chinese_Spring_v1....
chr5A_part2
chr5A_part2:134,193,724-134,193,798
Go
Switch the current genome
76 bp
134,193,730 bp
134,193,740 bp
134,193,750 bp
134,193,760 bp
OA.vcf
Galaxy713-[Gal...m.vcf].vcf.vcf
CL.vcf
Galaxy721-[Gala...am.vcf].vcf.vc
KSA.vcf
Galaxy761-[Gal...m.vcf].vcf.vcf
GT.vcf
Galaxy707-[Gal...m.vcf].vcf.vcf
HW.vcf
Galaxy605-[Gal...m.vcf].vcf.vcf
KSA.bam Coverage
KSA.bam
SNP linked to positive allele?
Lines that have positive allele for the gene

Extract context sequence and SNP

GTTAGGCCCTTTAGCTATCCAGGGGAACCCAATTGACTGATG
CATGTTTTAATCCAAAAAGGCCCTAGTCTAGTCATGCTGCTAG
CTGCATGCTAAAACTGAAGGGGACCTATTCATTCATTATCTAG
GGTCATTTACTTAAGGATACCTCATTATATATTATATTTTTGGCC
CCCATCGTCGTCTAA**[T/C]**AATCTGTCTAGTCTGTACGTCAACC
CAAAAGGTCTTGGGTTAACCCAGCTGTCAT

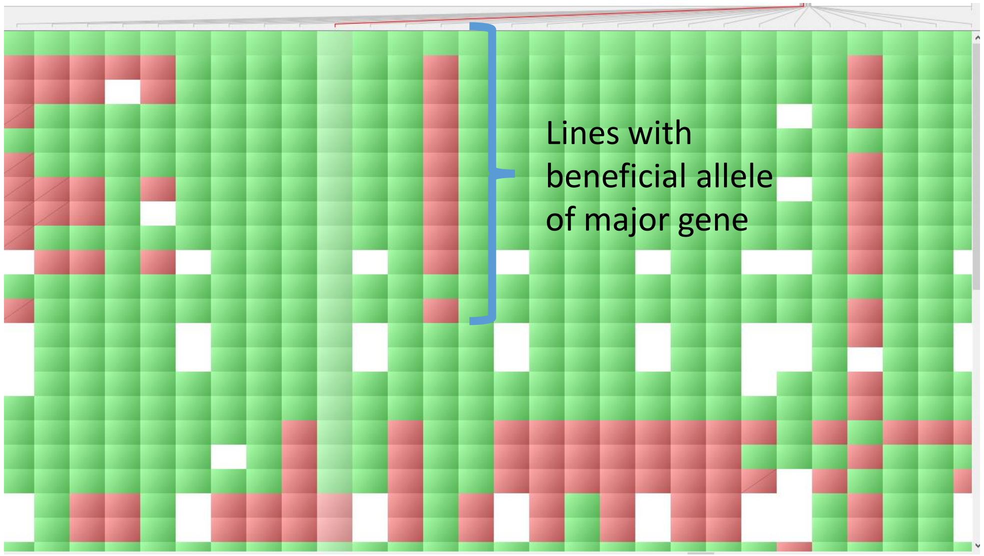
KASP Design

PolyMarker

TGAC The Genome Analysis Centre™ BBSRC

John Innes Centre Unlocking Nature's Diversity

Test KASPs on variety panels and match to known phenotype and/or presence of major gene



Marker diagnostic only in UK and German material.

SNP identified in UK material so linked to gene only in related germplasm?

Higher density SNP information would be beneficial in this case

WAKS in wheat

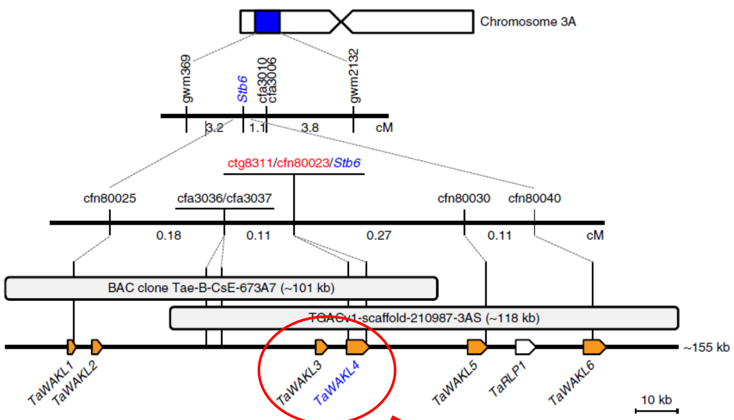
Thursday 14th February 2019

Kostya Kanyuka

Designing Future Wheat (WP2)

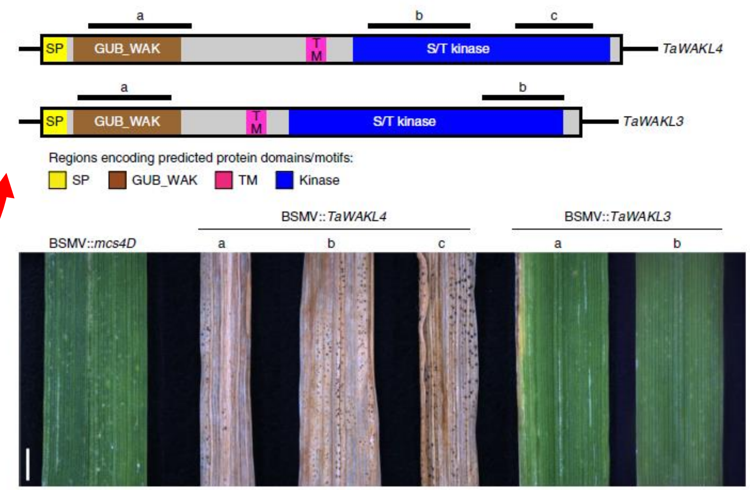
ROTHAMSTED RESEARCH

Map-based cloning of the first wheat gene – *Stb6* – for resistance to Septoria tritici blotch



Silencing of candidate genes using VIGS

TaWAKL4 = ***Stb6***



Stb6 wheat

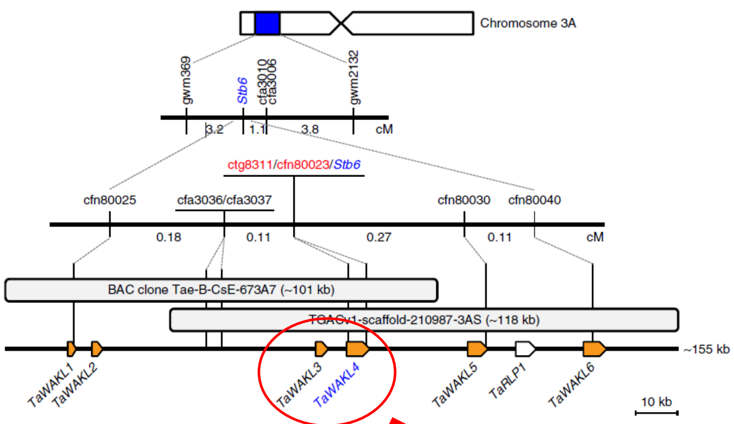
Nature Genetics (2018) 50: 368-74

collaboration with Cristobal Uauy (JIC) and Cyrille Saintenac (INRA)

BBSRC bioscience for the future

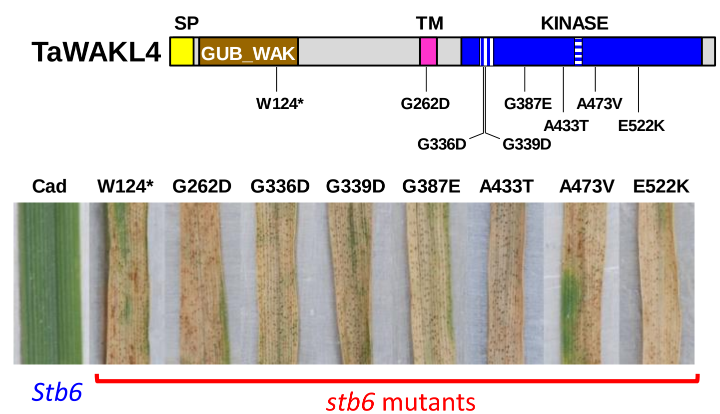
ROTHAMSTED RESEARCH

Map-based cloning of the first wheat gene – *Stb6* – for resistance to Septoria tritici blotch



Mutation analysis (TILLING) of candidate genes

TaWAKL4 = ***Stb6***



Nature Genetics (2018) 50: 368-74

collaboration with Cristobal Uauy (JIC) and Cyrille Saintenac (INRA)

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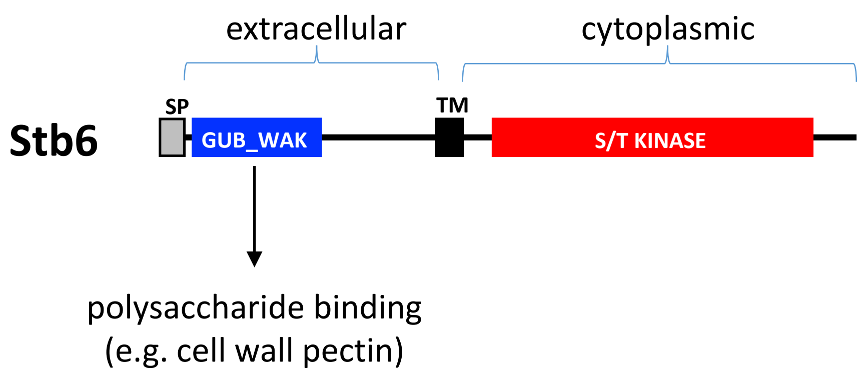
Designing Future Wheat (WP2)

ROTHAMSTED RESEARCH

Stb6 **encodes a Wall-associated kinase (WAK) protein: the first example of this class of extracellular receptors conferring gene-for-gene resistance to a pathogen**



collaboration with Cristobal Uauy (JIC) and Cyrille Saintenac (INRA)

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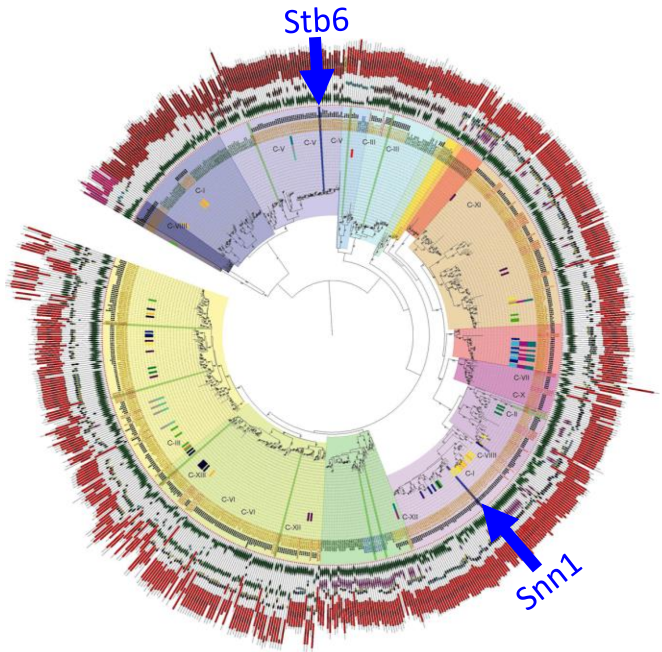
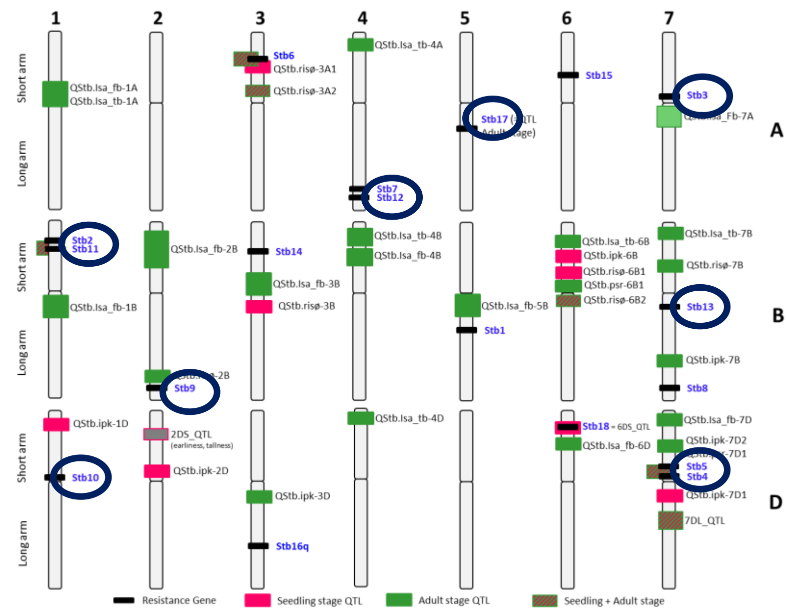
Designing Future Wheat (WP2)

Wheat genome contains over 600 WAK genes, some of which may correspond to other genetically defined Septoria resistance genes or confer resistance to other pathogens



Phylogenetic tree of 601 wheat WAKs

Septoria resistance (*Stb*) genes



collaboration with Cristobal Uauy (JIC) and Cyrille Saintenac (INRA)

	REGION	LEAD PI	CULTIVAR	PEDIGREE
De Novo Assembly	Australia	Langridge, U of A	Mace	Yuma//PI 372129/3/CO850034/4/4*Yuma/5/(KS91H184/Arlin S//KS91HW29/3/NE89526)
De Novo Assembly	Australia	Langridge, U of A	Lancer	VI184/Chara//Chara/3/Lang
De Novo Assembly	Canada	C. Pozniak, P. Hucl U of S, Andrew Sharpe - GIFS	CDC Landmark	CDC Teal//EE8/Kenyon35//AC Barrie
De Novo Assembly	Canada	C. Pozniak, P. Hucl U of S, Andrew Sharpe - GIFS	CDC Landmark	Unity/Waskada//Alsen/Superb
De Novo Assembly	Germany	N. Stein, K. Meyer IPK	Julius	Julius = Asketis x Drifter
De Novo Assembly	Japan	_	Norin61	_
De Novo Assembly	Switzerland	B. Keller, U of Zurich S. Krat Abdullah University	Arina	_
De Novo Assembly	USA	Poland, K State	Jagger	KS-82-W-418/STEPHENS
Assembly WR2AP	UK	BBSRC	Cadenza	Axona x Tonic, RBP95-73, Maris Dove, HPG-522-66
Assembly W2RAP	UK	BBSRC	Paragon	CSW-1724-19-5-69 x Axona/Tonic, introgression of diccocoides on 5B
Assembly W2RAP	UK	BBSRC	Kronos	_
Assembly W2RAP	UK	BBSRC	Robigus	1366 x Z-836
Assembly W2RAP	UK	BBSRC	Claire	Flame x Wasp,/Moulin/Taurus/Boxer/Galahad