Wheat
Genetic
Improvement
Network

John Innes Centre

Tools, resources, genotyping and phenotyping

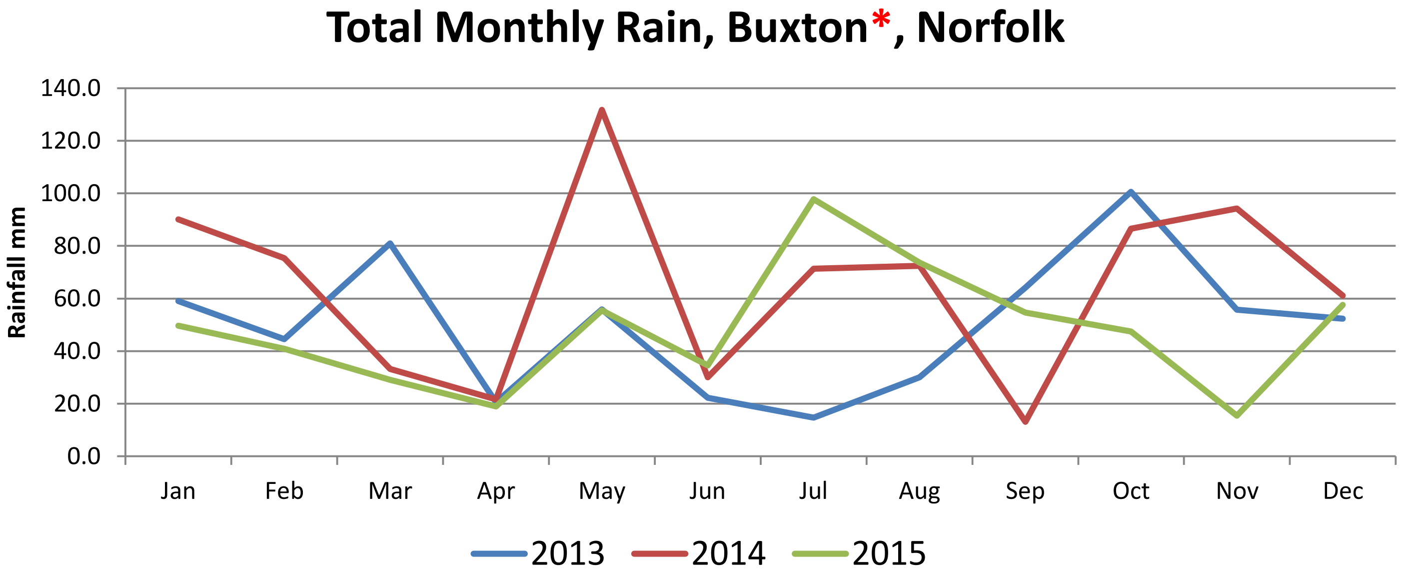
Clare Lister
02/03/2016

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

1. UK drought tolerance in Paragon x Garcia

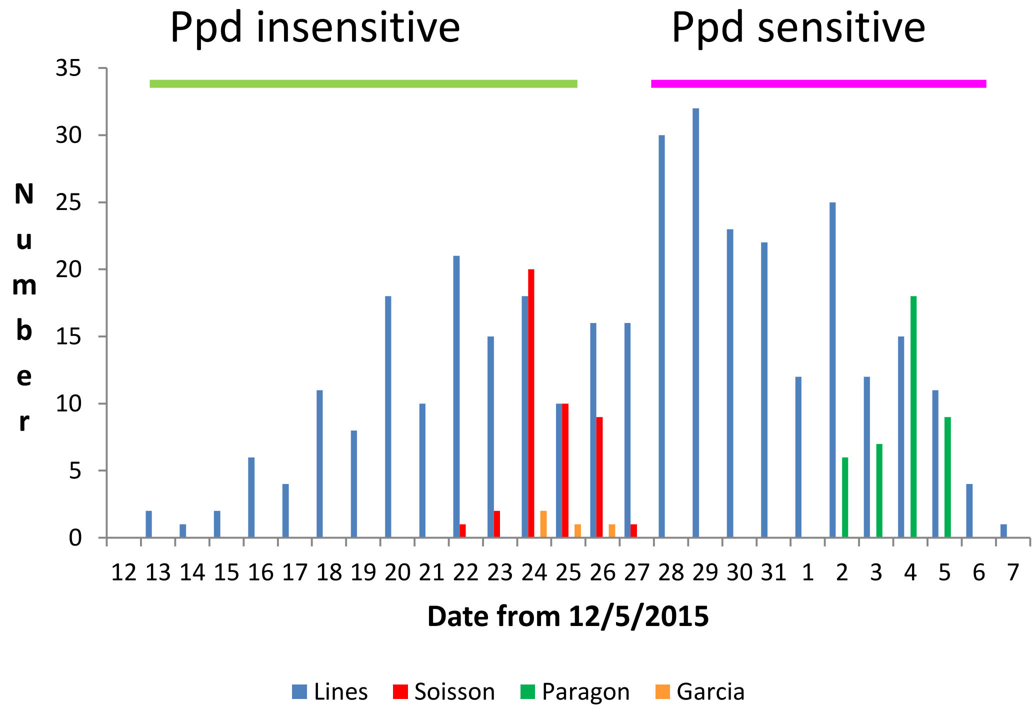
- Paragon x Garcia (PxG) RIL population produced within WGIN to specifically target UK drought
- Paragon (UK spring wheat) x Garcia (grown in southern France and northern Spain, adapted to drought stress).
- Looking for drought-tolerant characteristics in non-UK wheat varieties highly advantageous ->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


Total Monthly Rain, Buxton*, Norfolk

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

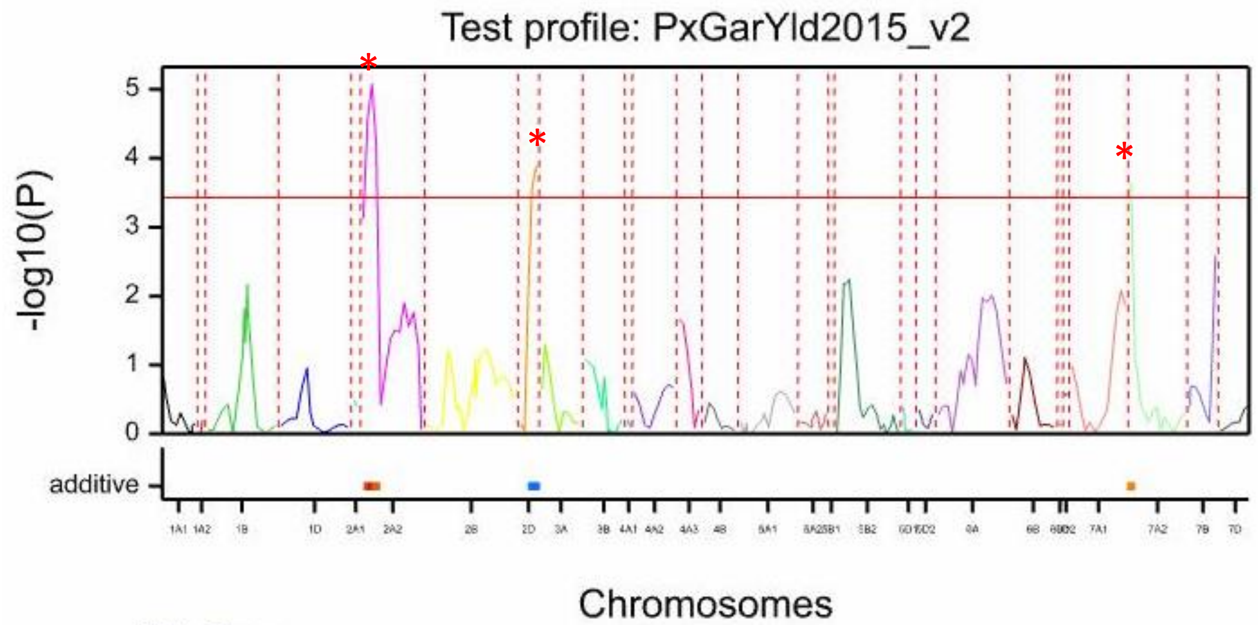
1. UK drought tolerance in Paragon x Garcia

- Single-rep 1m field trial 2014-15
- F6 scored for DTEM, Height, Yield and TGWT*
- QTL mapping (KASP map) using Single-Trait Linkage Analysis
- QTLs identified for all traits scored

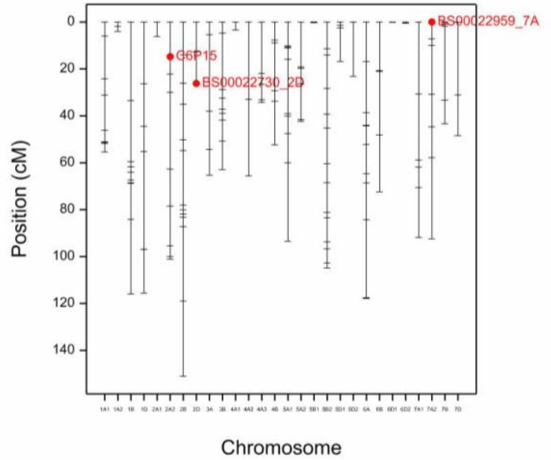


1. UK drought tolerance in Paragon x Garcia

Yield



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



QTL effects

Locus no.	Locus name	%Expl. Var.	Add. eff.	High value allele	s.e.
55	C6P15_2A	8.468	0.076	Garcia	0.017
97	BS00022730_2D	4.079	0.053	Paragon	0.014
257	BS00022959_7A	4.046	0.052	Garcia	0.014

Candidates?

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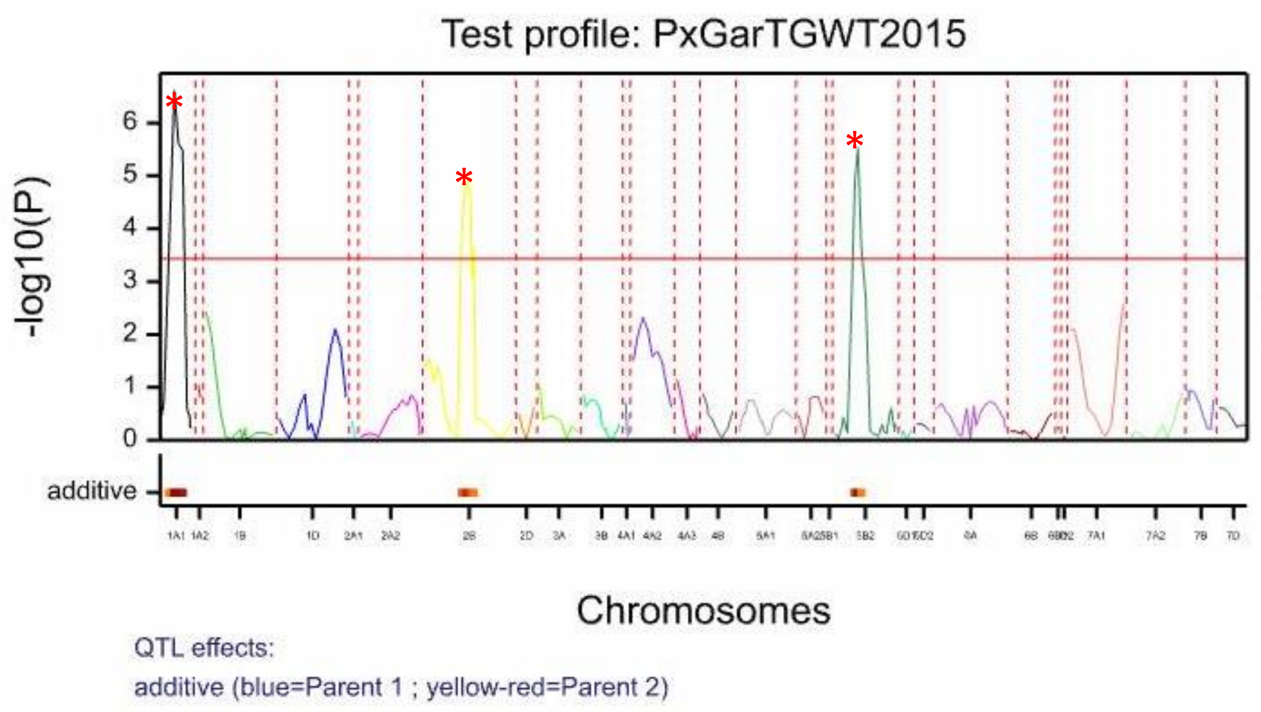
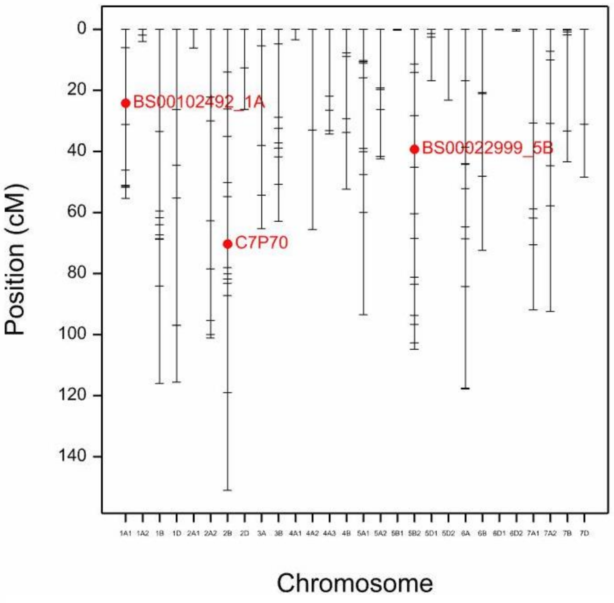
1. UK drought tolerance in Paragon x Garcia

TGWT*


Test profile: PxGarTGWT2015

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



QTL effects

Locus no.	Locus name	%Expl. Var.	Add. eff.	High value allele	s.e.
4	BS00102492_1A	11.708	1.528	Garcia	0.296
78	C7P70_2B	14.040	1.674	Garcia	0.379
185	BS00022999_5B	9.784	1.397	Garcia	0.299

Candidates?

1. UK drought tolerance in Paragon x Garcia

- Drought Trial drilled with selected P x Gar lines (Ppd-sensitive)
- Also included subset of Paragon Library in trial
- 2 reps each **Irrigated** and **No Irrigation**
- 200 lines/rep, 6 square meter plots

No Irrigation

Irrigated with boom

Slope

1. UK drought tolerance in Paragon x Garcia

- Will measure stage 31 (P x Gar)
- Will measure DTEM, Booting, Height, Yield, TGWT and calculate grains/m^2
- Monitor/record drought-recovery strategies
- Photo of each plot
- Periodic imaging of plots during growth to track senescence, using UAV

Drought Tolerance

- To correlate the response of the plants to conditions during the growing season data will be collected from one **irrigated** **Paragon** plot and one **Paragon** plot **without additional irrigation**

- Will measure soil temperature, soil water content and water potential
- Weather data for Church Farm plots will also be available
- Using monitoring equipment from **DeltaT**

- Collaborating with John Foulkes (Uni Nott.)
- 2D root screening to characterise variation in key root architectural traits (including root length, angle, and distribution)

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

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- Winter-sown, single rep, yield trials of NILs carrying multiple alleles of Rht’s, Ppd’s, Vrn’s, eps, grain shape, yield…
- DTEM, Height, Yield and TGWT scored

- 3 rep, spring-sown, yield-trial of **subset** of Paragon Library
- DTEM, Height, Yield and TGWT scored

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

Paragon	Par Library	
Lr19 Kamb1	Par Library 2-15	Alien introgression*
Par Mutant 2316b	Par Library 20-9	Staygreen
Ppd 1x Early	Par Library 15-12	DTEM
Ppd 2x Early	Par library 16-7	DTEM
Ppd 3x Early	Par library 16-15	DTEM
Ppd KO 3x	Par Library 19-1	DTEM
Rht 8 Mara	H14 Nor N Med Irr-9	Height
Rht B1 Robigus	Par library 3-20	Height
Rht D1 Alchemy	Par Library 3-12	Height

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

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

- Paragon Library subset yield trial repeated
- Also included in Drought Trial
- 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 etc… will be scored

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

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

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

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

- AFM (in WGIN) made a winter Paragon by crossing in *VrnA1* + *VrnB1* from Malacca
- Will cross this into *Rht8* / *RhtB1* / *RhtD1* to produce winter, semi-dwarf, Paragon

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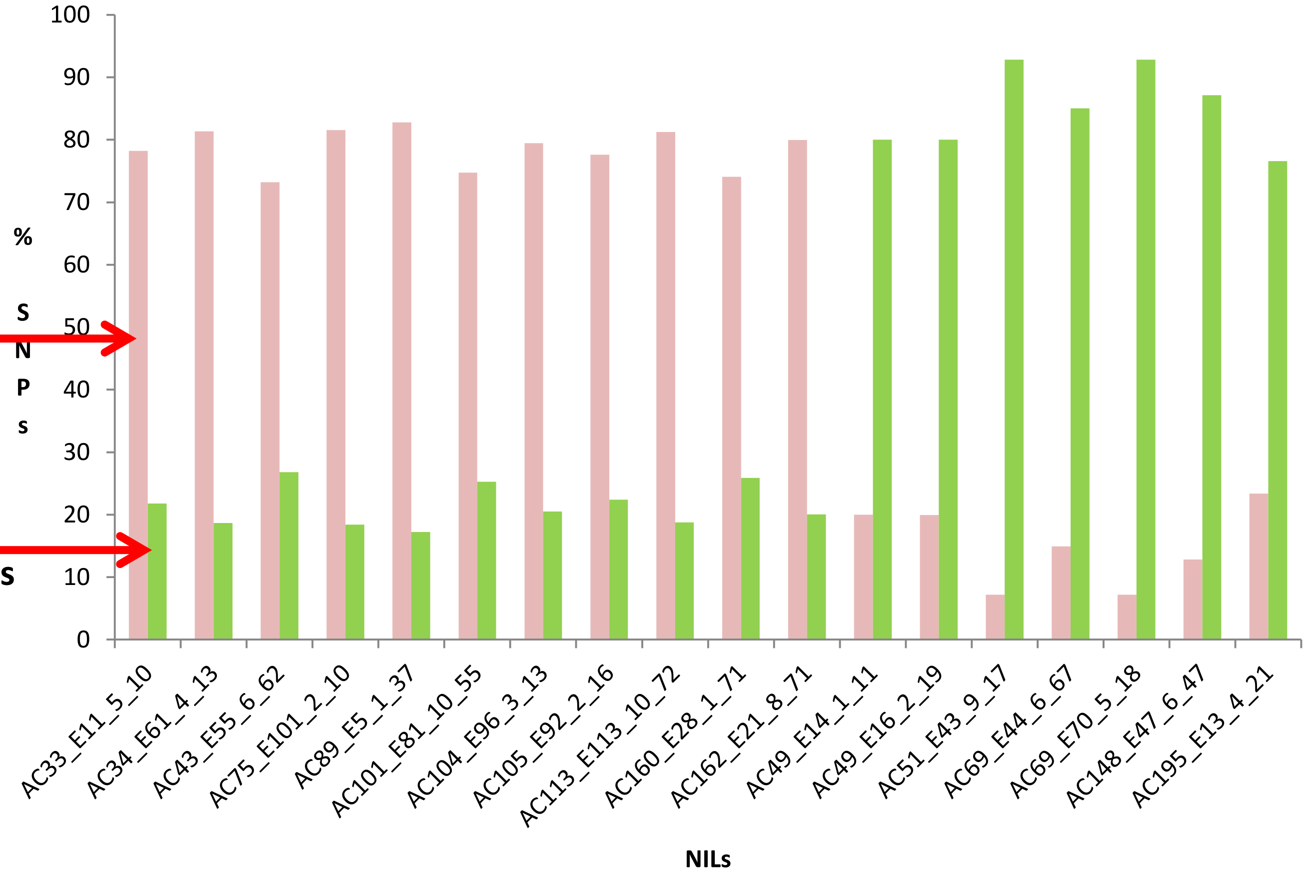
A chromosome segment substitution library (CSSL) for Avalon x Cadenza

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

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

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



i.e.
~78% Cadenza background

~22% Avalon
QTL region + random segments

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.

QTL region - Avalon

Random background - Avalon

Cadenza

X

Individual 1

Individual 2

Individual 3

i.e. A genome

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

- We needed to generate genetic maps of all the NILs to determine the extent and location of the QTL regions and random segments

- This has been carried out 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

- The maps are now available on the WGIN website http://www.wgin.org.uk/

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

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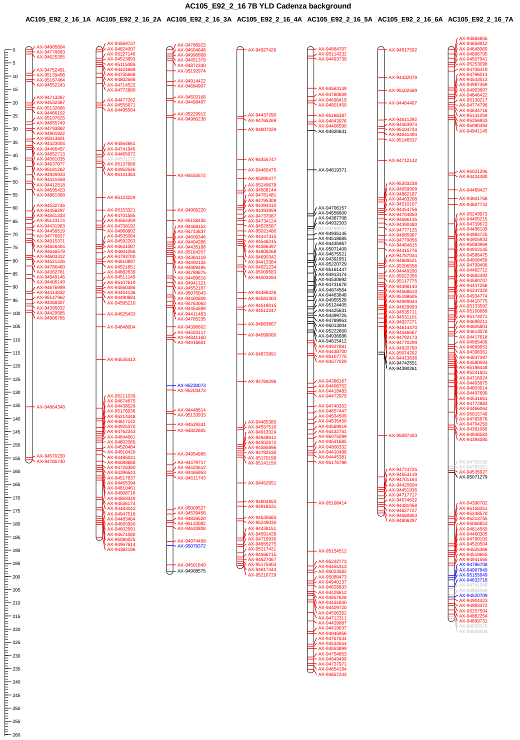
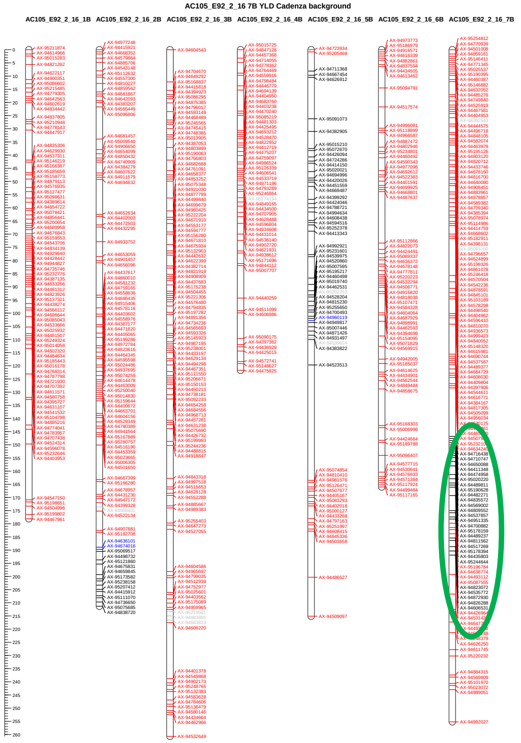
4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza

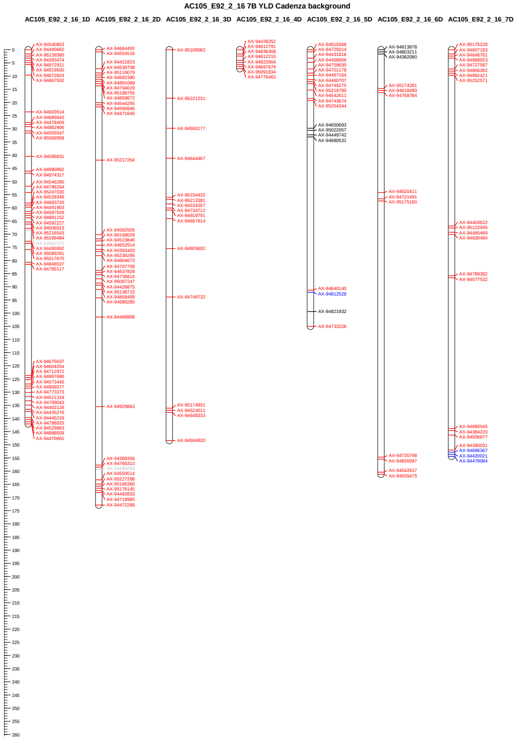
A Genome



B Genome



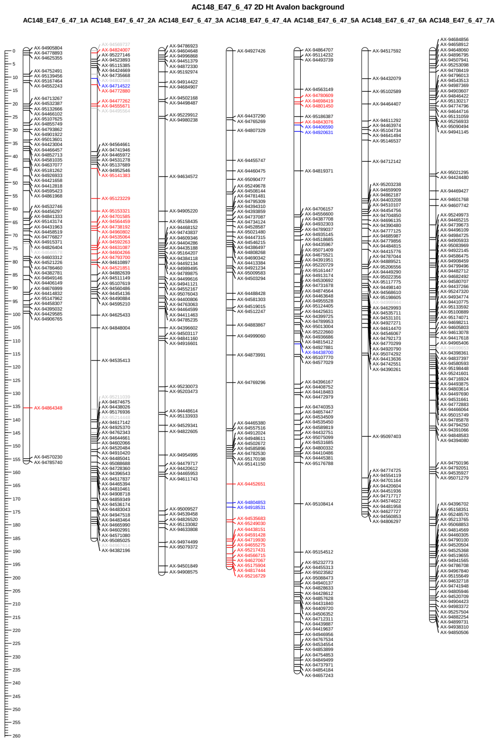
D Genome



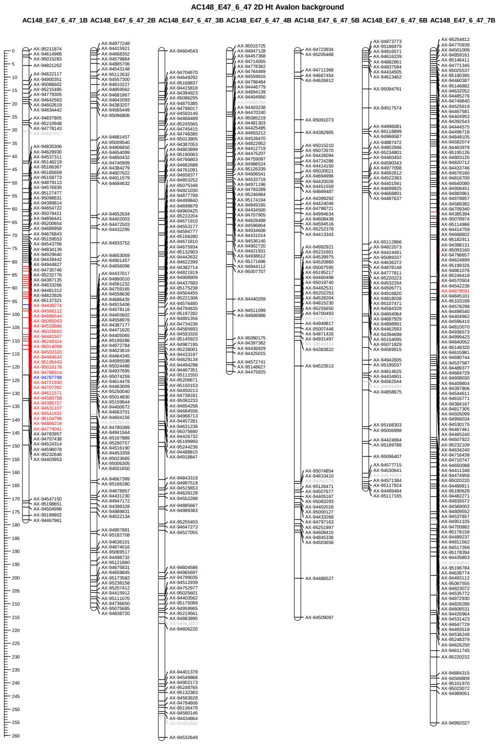
Cadenza background - **Avalon** *random* background and **QTL region**

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

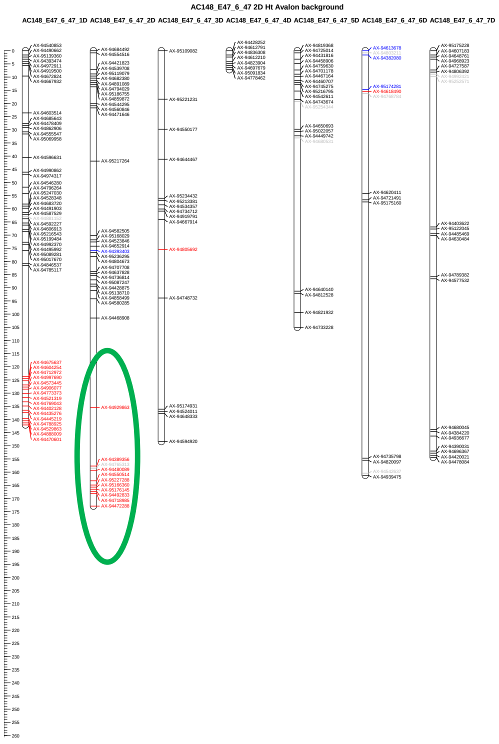
A Genome



B Genome



D Genome



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

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

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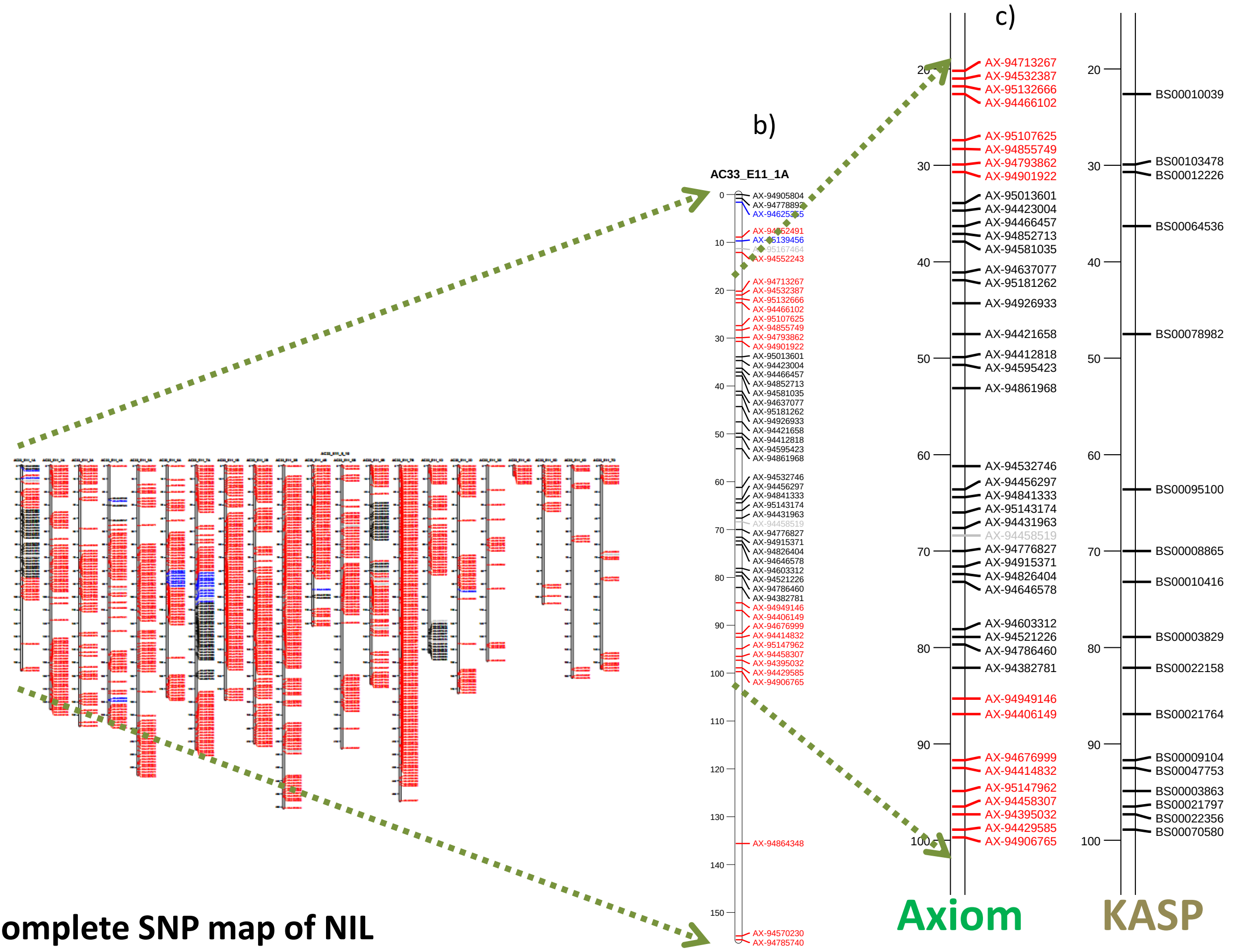

Complete SNP map of NIL
With QTL for 1D DTEM

Chromosome 1A

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

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

- The next stage involves genotyping another 94 lines representing the QTLs in the AxC NILs (DTEM, Ht, YLD)

Background	Chromosome	Trait	QTL Allele	# of lines
Avalon	1B	EM	Cadenza	5
Avalon	1D	EM	Cadenza	3
Avalon	2A	Ht	Cadenza	2
Avalon	2D	Ht	Cadenza	5
Avalon	2D	YLD	Cadenza	3
Avalon	3A	Ht	Cadenza	5
Avalon	3B	Ht	Cadenza	5
Avalon	5A	YLD	Cadenza	5
Avalon	6A	Ht	Cadenza	5
Avalon	6B	Ht	Cadenza	5
Avalon	7B	YLD	Cadenza	1
Avalon	7D	YLD	Cadenza	3

Background	Chromosome	Trait	QTL Allele	# of lines
Cadenza	1B	EM	Avalon	5
Cadenza	1D	EM	Avalon	5
Cadenza	2A	Ht	Avalon	5
Cadenza	2D	Ht	Avalon	6
Cadenza	3A	Ht	Avalon	6
Cadenza	3B	Ht	Avalon	5
Cadenza	3B	YLD	Avalon	5
Cadenza	6A	Ht	Avalon	5
Cadenza	6B	EM & Ht	Avalon	5

- The 18 + 94 NILs are being backcrossed to the recurrent parent twice to generate lines in which the QTL region and random segments have been separated.

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

- Update on backcrosses to recurrent parents

	Avalon Background	Cadenza Background
Crosses done	23	28
Crosses successful	8 BC1	12 BC1
Previous crosses (CJM)	7 BC1	3 BC2

- Will genotype BC1 progeny, with parent line, to confirm crosses before proceeding

- 94 DNAs ready for genotyping
- Arrays and reagents already at Bristol
- These data will allow prioritization of future crosses

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7. Applying WGIN data to breeding by design for UK yield stability CROSSES & SEED BULKING UNDERWAY (SEO)
8. Curation and distribution of WGIN germplasm ONGOING

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8. Curation and distribution of WGIN germplasm ONGOING

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	Cordialle	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
Cordialle	x	Revelation	heading	brown rust		y
Cordialle	x	Crusoe	GS31	market share		y
Cordialle	x	Alchemy	height			y
Cordialle	x	Invicta	heading			y
Conqueror	x	Scout	lodging	eyespot		y
Claire	x	Revelation	yield untr			y

WGIN3 Management Meeting

2nd March 2016

Screening germplasm for resilience to aphids (WP2.3)

Lesley Smart

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WISP

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bioscience for the future

ROTHAMSTED
RESEARCH

The Target Pests

Rhopalosiphum padi

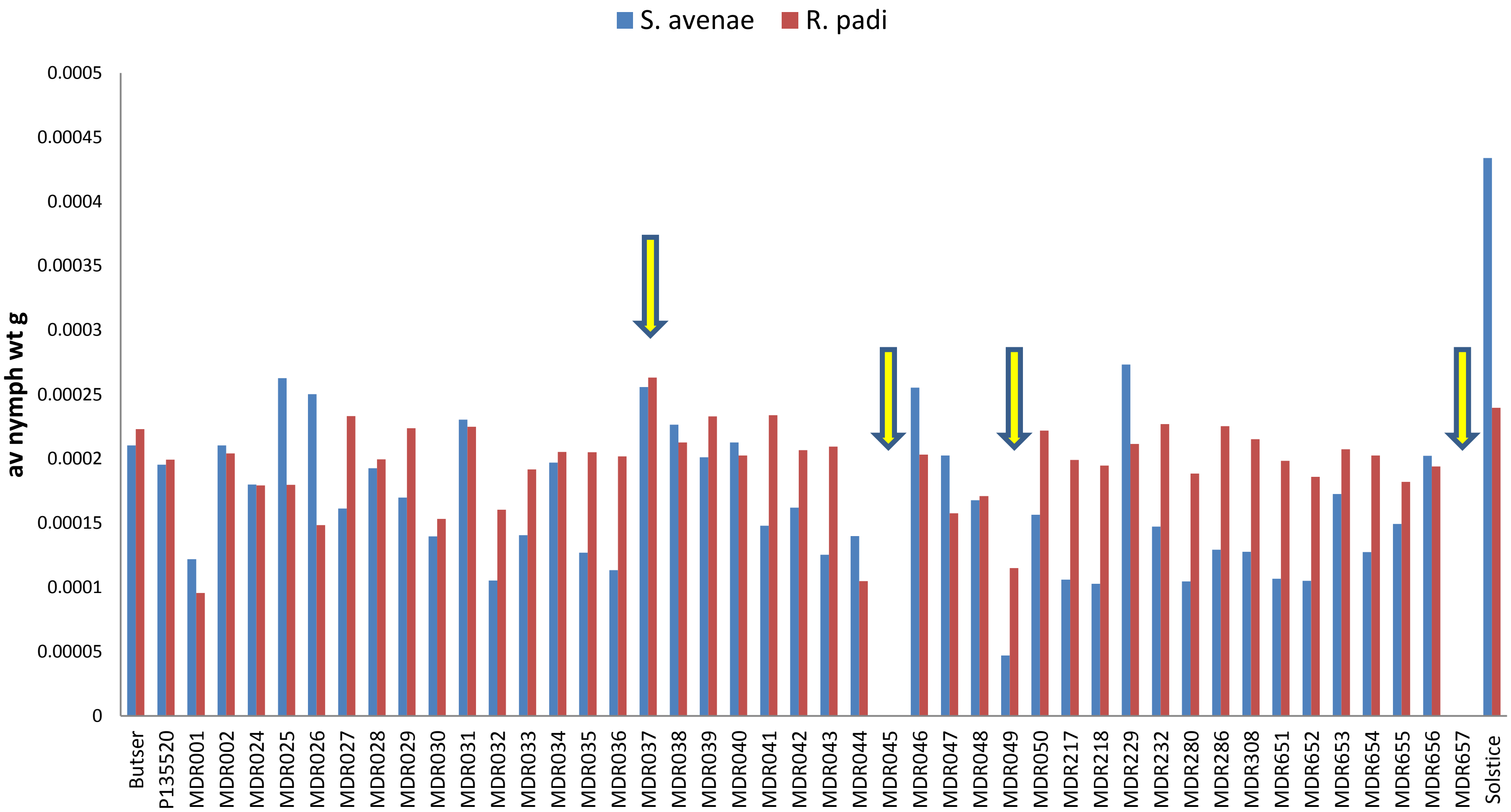
Sitobion avenae

Screening germplasm for resilience to aphids (WP2.3)

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

- Focus on some *Triticum monococcum* lines as these provided the most promising leads for partial resistance to cereal aphids from work in WISP
  - Fewer if any 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

Nymph weight on *Triticum monococcum* lines



WISP

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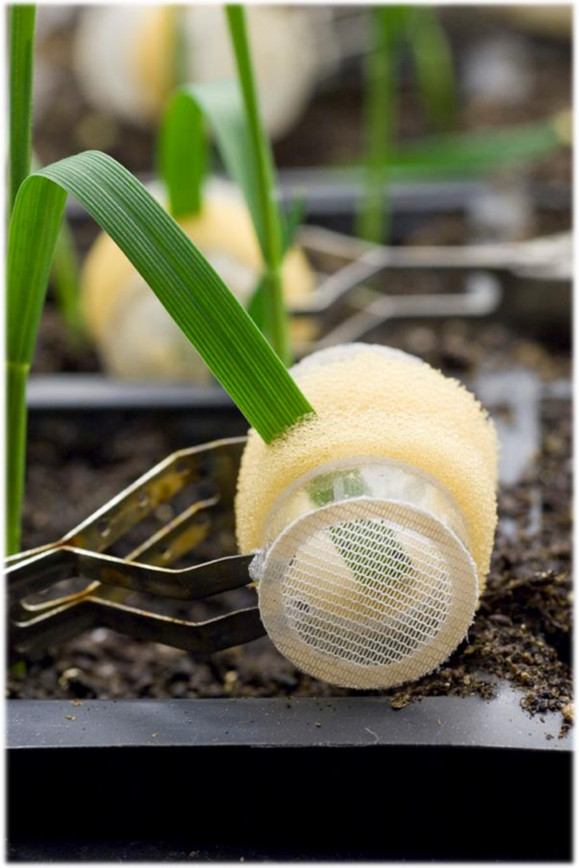
RESEARCH

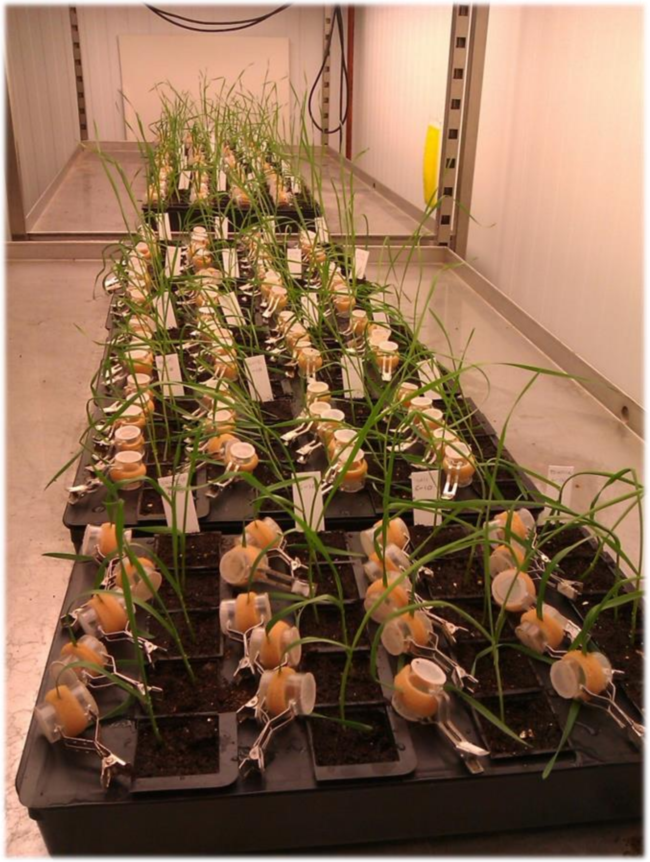
Screening germplasm for resilience to aphids (WP2.3)

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

- Crosses made by Mike Hammond-Kosack: MDR037 (susceptible) x MDR045, MDR049 and MDR657 (all showing partial resistance)
- F1 generations of these crosses have been tested in the phenotyping screen along with parental lines against both aphid species
- Differences observed between responses of aphid species to F1 generations in phenotyping screen, but data limited. Aphid response on some parental lines differed from original findings. MDR049 consistent reduction in nymph weight gain.
- F2 generations have now been tested in the phenotyping screen

- F2 generations of crosses, MDR037 x MDR045, MDR049 and MDR657, tested in the phenotyping screen against both aphid species





Where germination sufficient, 30 plants per cross in each trial with 20-30 Solstice plants. 4 crosses each for MDR037 x MDR657 and MDR049 and 3 crosses for MDR037 x MDR045

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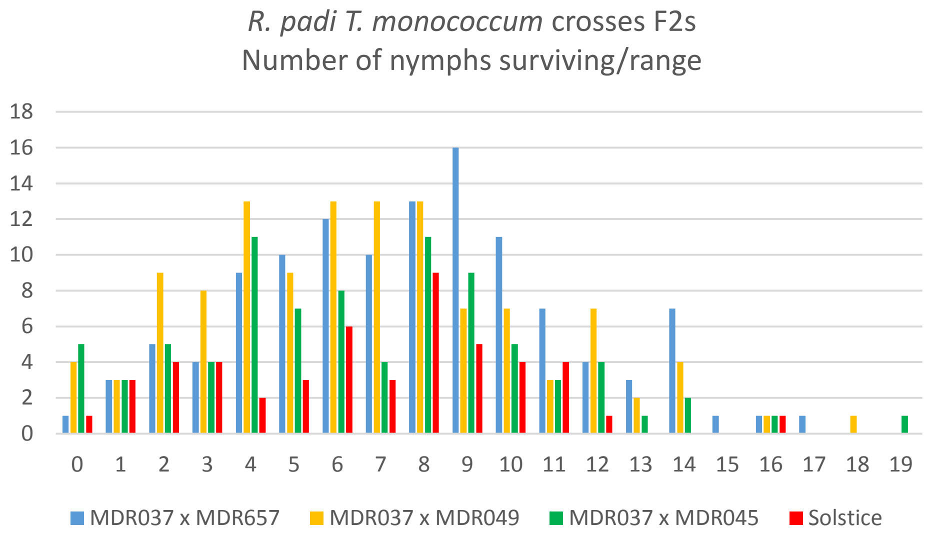
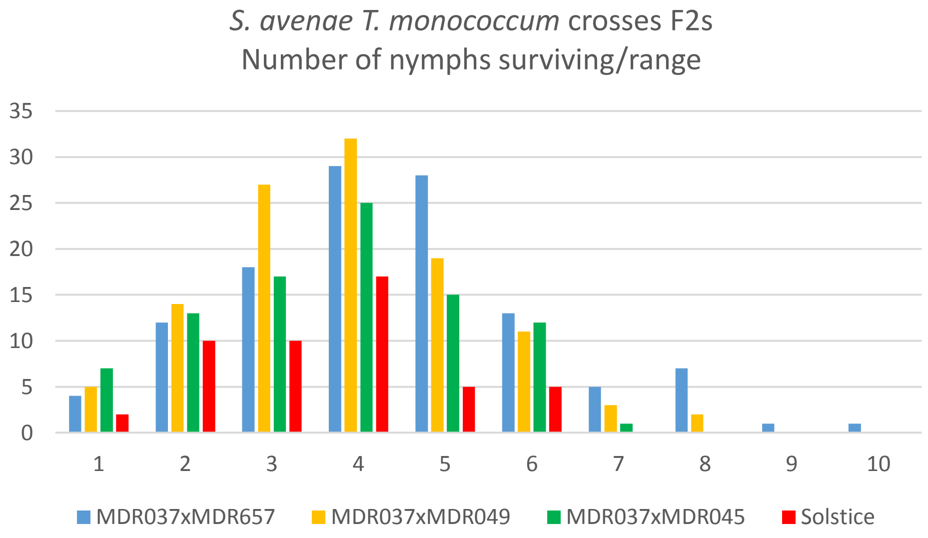
Nymph Production/ Survival

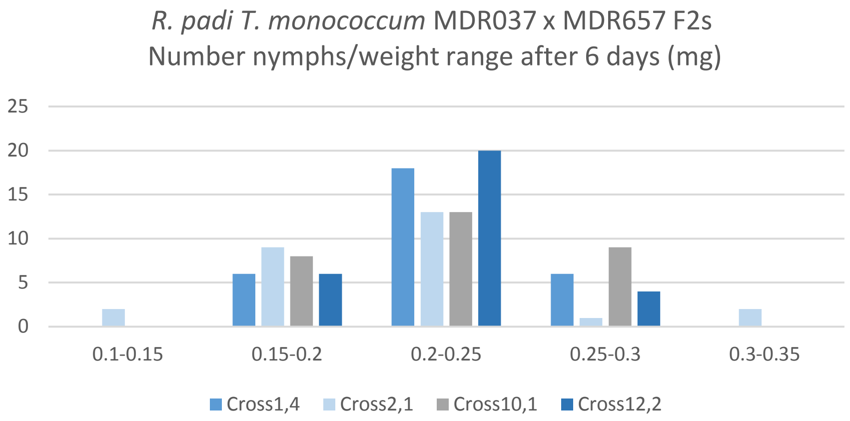

R. padi T. monococcum crosses F2s
Number of nymphs surviving/range

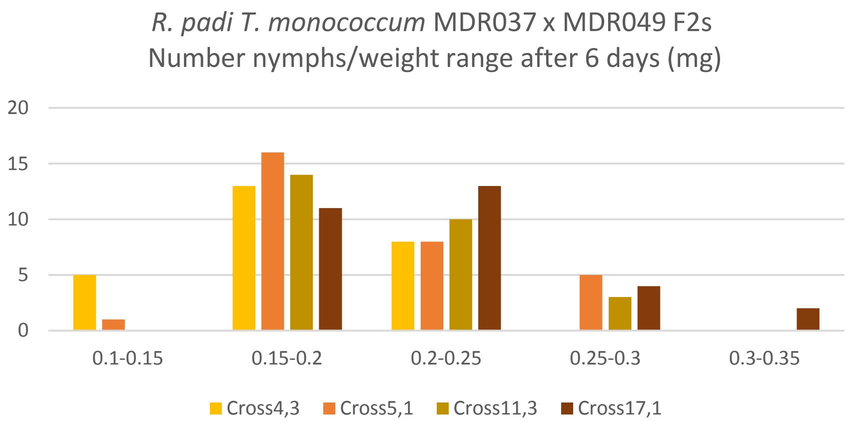

S. avenae T. monococcum crosses F2s
Number of nymphs surviving/range

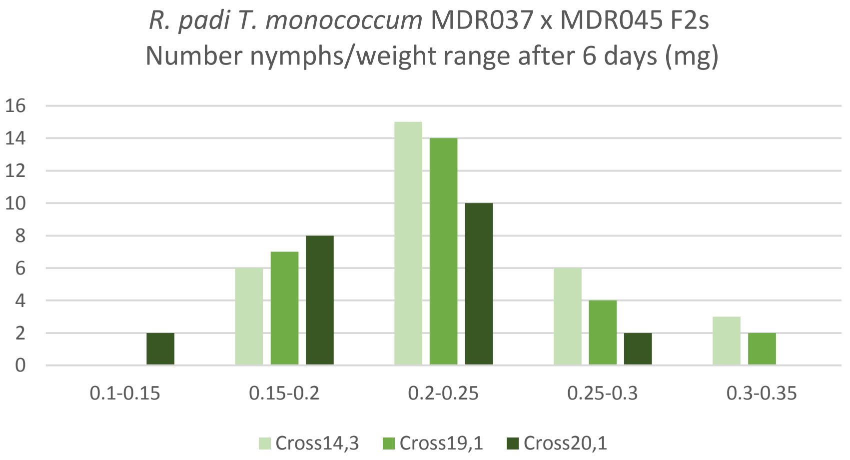
Little evidence of effect on nymph production for MDR037 x MDR657 or MDR045 as compared to parental lines where few if any nymphs produced by either species in previous trials.

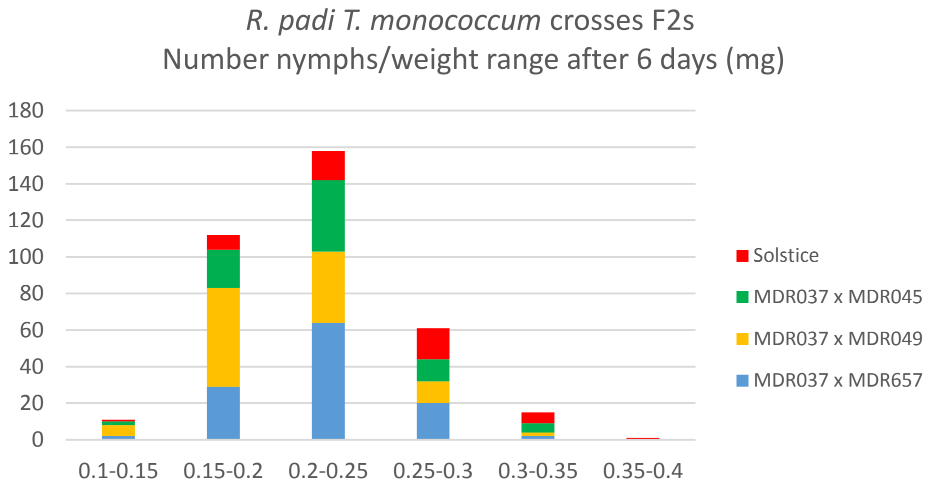
Replication
37x657 = 118
37x49 = 113
37x45 = 90
Solstice = 49

Frequency of *R. padi* nymphs in different weight ranges after 6 days on *T. monococcum* crosses at F2. (Nymphs produced over 24h period).


R. padi T. monococcum MDR037 x MDR657 F2s
Number nymphs/weight range after 6 days (mg)

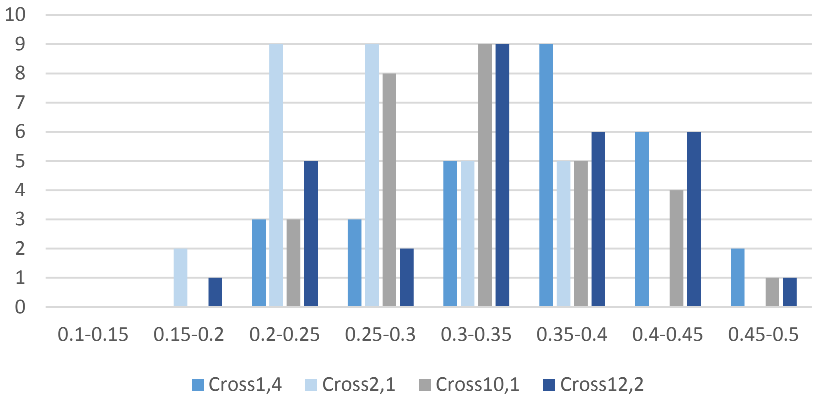

R. padi T. monococcum MDR037 x MDR049 F2s
Number nymphs/weight range after 6 days (mg)

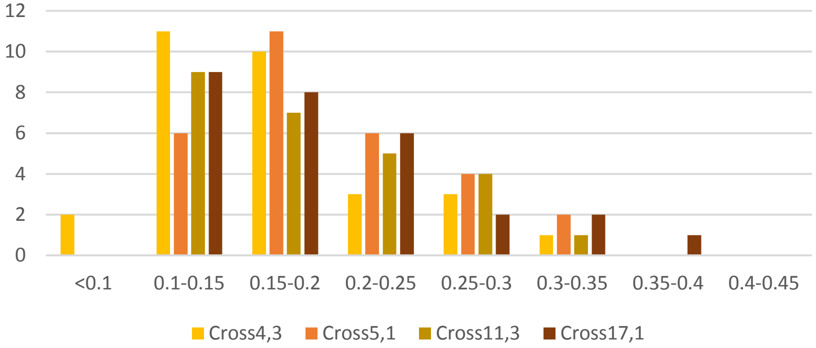

R. padi T. monococcum MDR037 x MDR045 F2s
Number nymphs/weight range after 6 days (mg)

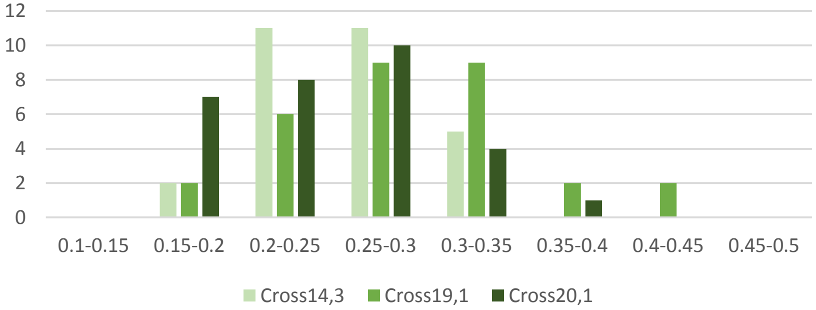

R. padi T. monococcum crosses F2s
Number nymphs/weight range after 6 days (mg)

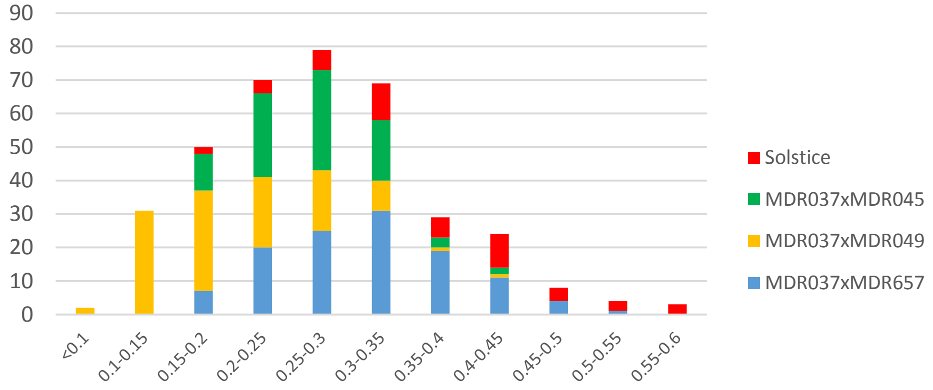
Plant no. 37x657 = 117; 37x49 = 113; 37x45 = 79; Solstice = 49

Frequency of *S. avenae* nymphs in different weight ranges after 7 days on *T. monococcum* crosses at F2. (Nymphs produced over a 48h period).


S. avenae T. monococcum MDR037 x MDR657 F2s
Number nymphs/weight range at 7 days (mg)


S. avenae T. monococcum MDR037 x MDR049 F2s
Number nymphs/weight range at 7 days (mg)


S. avenae T. monococcum MDR037 x MDR045 F2s
Number nymphs/weight range at 7 days (mg)


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

Plant no. 37x657 = 118; 37x49 = 113; 37x45 = 89; Solstice = 49

Summary

- F2 generations have been screened against both aphid species
- Some MDR037 x MDR049 F2s showing reduction in nymph weight gain, particularly for *S. avenae*. Little evidence of effects on nymph production.
- Plants currently in vernalisation for F3 generation

Rothamsted Research
where knowledge grows

WGIN 3
Resistance to take-all and foliar diseases

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Resistance to take-all and foliar diseases

Objectives:

1. **Complete development of *Triticum monococcum* mapping populations for genetic analysis of resistance to take-all**

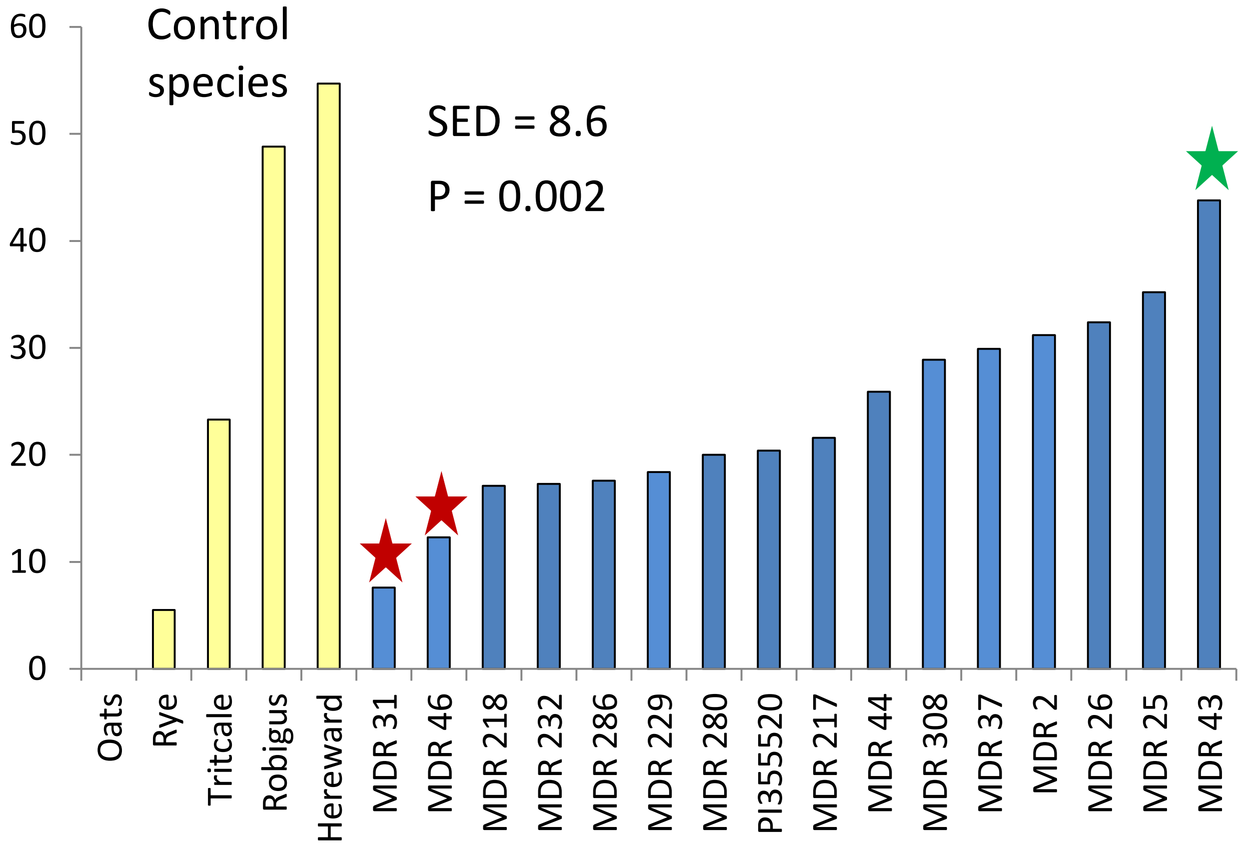
2. Continue the introgression of resistance to take-all from *T. monococcum* to the BC1 stage

3. **Examine the resistance of *Triticum monococcum* to yellow rust**

4. **Characterise hexaploid wheat germplasm previously shown to exhibit a high level of resistance to multiple foliar diseases**

Resistance to take-all in *Triticum monococcum*

- 3rd wheat field trials 2006-2011 (WGIN 1 and 2)
- 34 *T. monococcum* accessions tested over 5 years



McMillan et al. (2014) BMC Plant Biology 14: 212

Objective 1: Complete development of *Triticum monococcum* mapping populations

F_6 populations:

MDR037 (S) x MDR046 (R) – 79 F6 lines (started with ~180 F3 plants)

MDR037 x MDR229 – 85 F6 lines

F_2 *Tm* cross progeny numbers:

Parentage		Estimated F_3 progeny number
MDR031 (R) x MDR043 (vS)	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
MDR043 (vS) x MDR046 (R)	36 ears from 3 plants	750 ←
MDR229 x MDR031	94 ears from 6 plants	2000

Now at F_5, taking forward to F_6 for field trialling ←

Field trial screening MDR037 (S) X MDR046 (R) *T. monococcum* mapping population

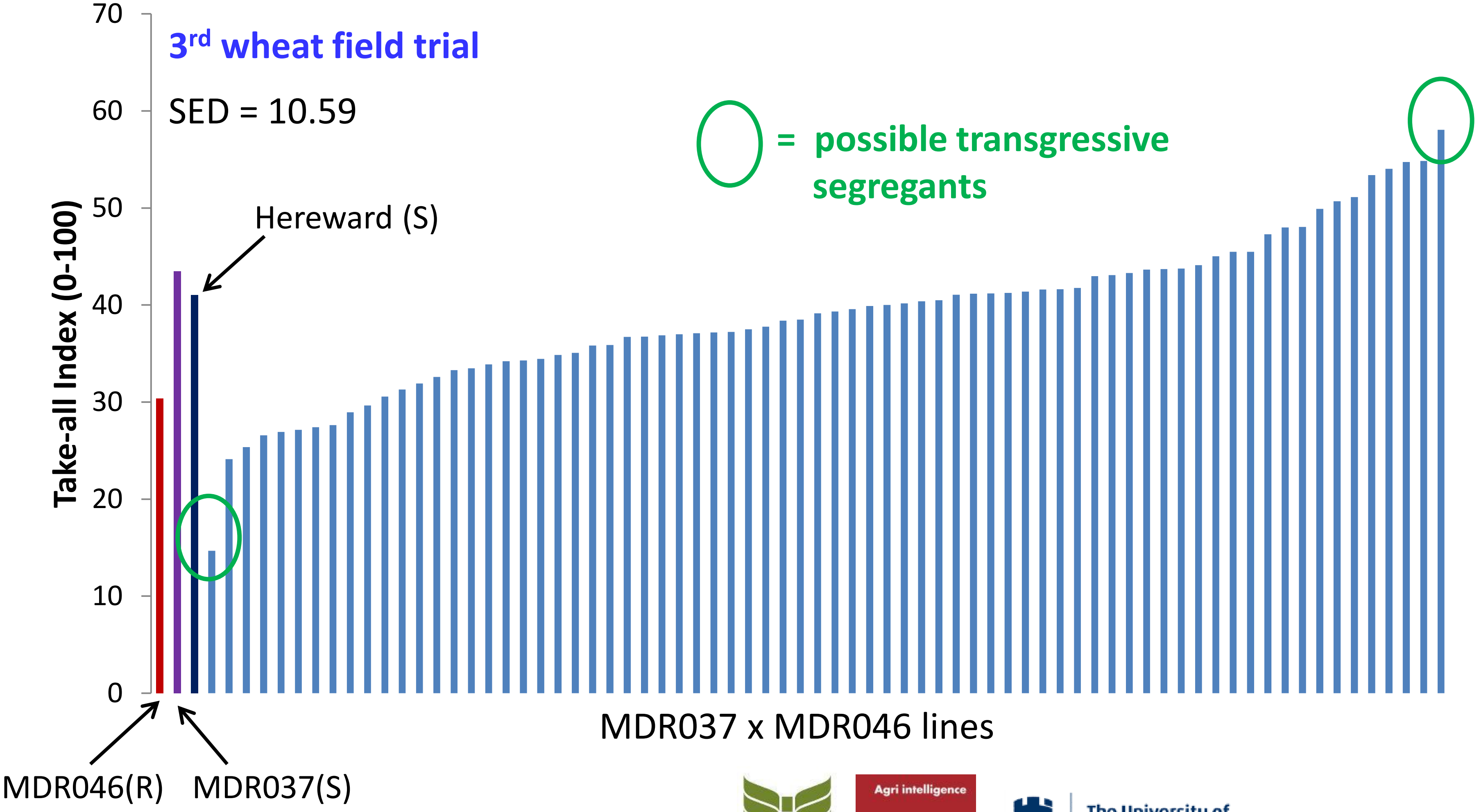
2013/2014 field trial (3rd wheat situation):

- Randomised block design (2-5 replicates/genotype)
- F_6 mapping population of 72 lines + parental lines

- Plant samples taken at GS 75

PhD student Sarah-Jane Osborne

MDR037 (S) x MDR046 (R) mapping population



PhD student Sarah-Jane Osborne

MDR037 (S) x MDR046 (R) mapping population

Correlations between take-all severity and above ground phenotypes

Spearman's Rank Correlation	Plant height (cm)	Days to anthesis from 1st May	Days to leaf senescence from 1st July
Rs	0.117	0.017	**-0.321**
P	0.319	0.982	**0.005**

- Earlier senescing lines had more severe take-all root infection
- Repeat field trial 2014/2015 – currently being assessed
- Population currently being genotyped at Bristol

Resistance to take-all and foliar diseases

3. Examine the resistance of *T. monococcum* to yellow rust

- Non-host/highly resistant across whole collection in 2015 inoculated field trial (n = 216 accessions)
- Collection to be genotyped to provide fully characterised genetic resource for future studies

Spring habit	172
Winter habit	37
Country of origin	29

- Will now focus on more promising Watkins lines for characterising foliar disease resistance (see objective 4)

Resistance to take-all and foliar diseases

4. Characterise hexaploid wheat germplasm previously shown to exhibit a high level of resistance to multiple foliar diseases

Background Watkins 2008 Field Trial

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?

WGIN 3 Watkins foliar disease experiment 2015

- 10 Watkins lines + controls sown in both **1st wheat (no take-all)** and **3rd wheat (high take-all)** field trials in autumn 2014 (1 or 2 replicates per line in each trial)
- No fungicides applied to allow natural disease to develop
- Score for foliar diseases + take-all

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

			2008 Disease assessments				
Accesssion	Growth habit	Country of Origin	Yellow rust	Brown rust	Septoria	Mildew	Mapping population at JIC
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	YES - with Paragon
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	

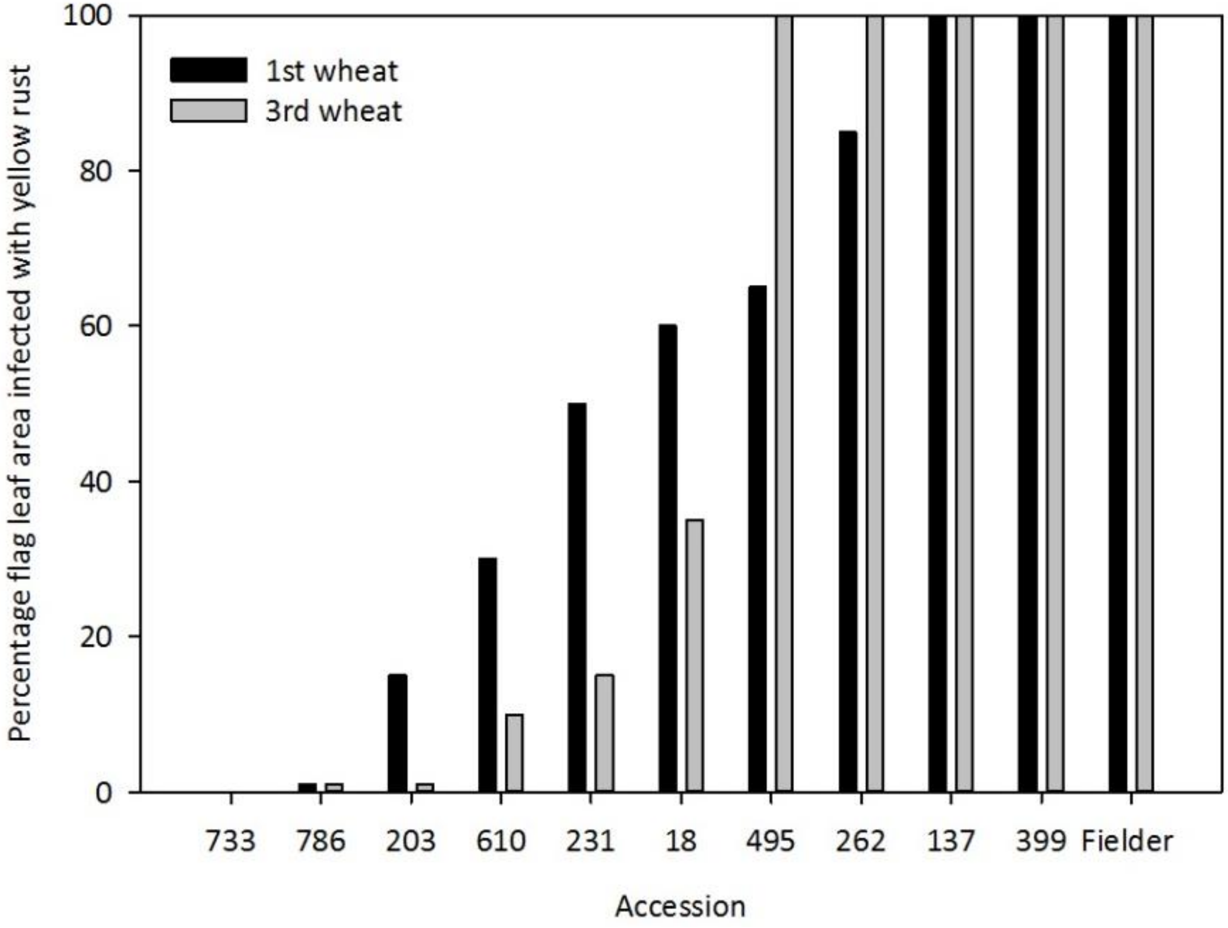
0 – no disease , T = trace

Watkins foliar disease field trial 2015

26th June 2015 1st wheat Long Hoos 4

Yellow rust dominant disease that developed across 2015 field trials

Evidence of resistance to yellow rust



- Evidence of induced response due to take-all?
- Take-all disease assessments and full statistical analyses to be completed

Evidence of resistance to yellow rust

Watkins 203

Watkins 733

Repeat field trial drilled in autumn 2015

Powdery mildew, Septoria leaf blotch and yellow rust already visible on farm

Watkins mapping population development

Field crossing with cv. Fielder Summer 2015

Accession Number	Growth habit	Country of Origin	Ears crossed	F_1 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

- Mapping populations have been sown in glasshouse to generate F2 and for backcrossing to cv. Fielder
- Watkins 786 x Fielder crossing to be carried out in glasshouse
- F_1 grain also to be included in a spring field trial 2016 to study inheritance

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