Wheat
Genetic
Improvement
Network

John Innes Centre

Tools, resources, genotyping and phenotyping

Clare Lister
28/10/2015

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WGIN3 Projects: Griffiths' Lab

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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

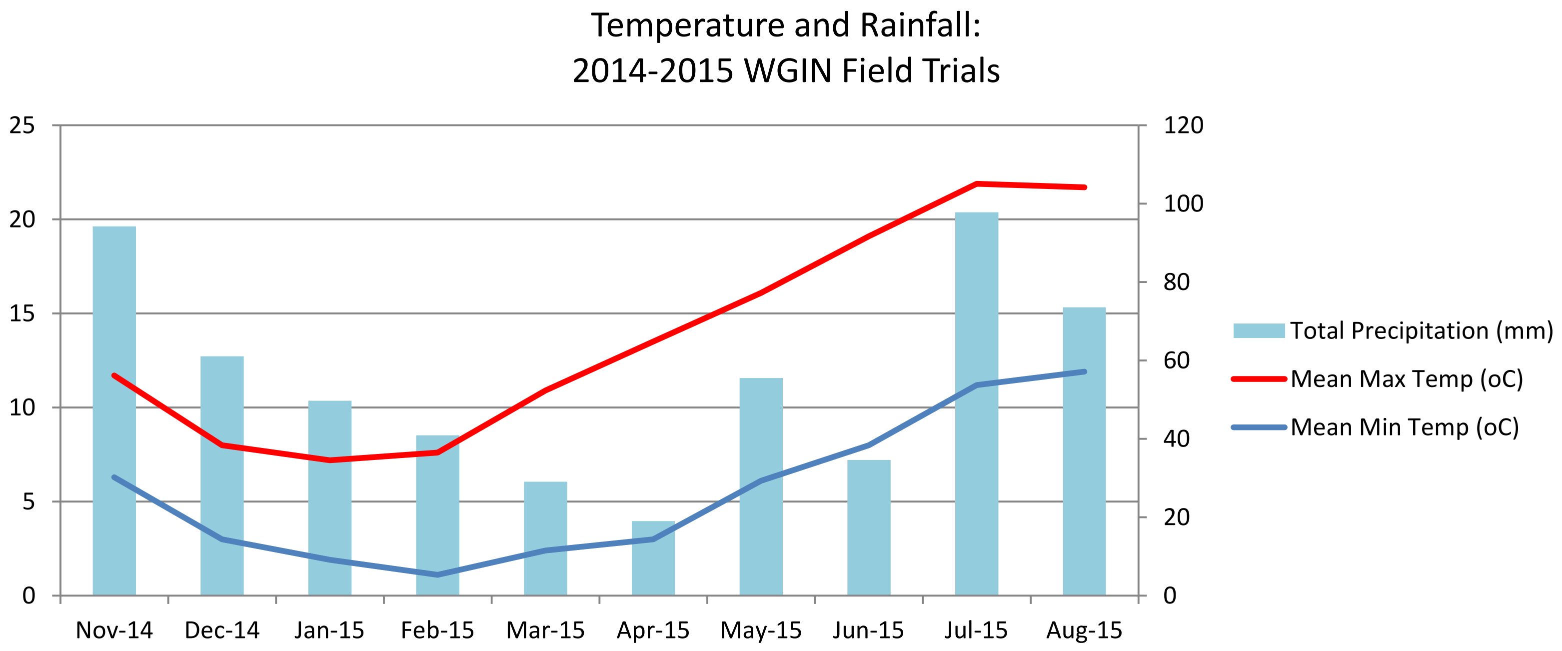
WGIN3 Projects: Griffiths' Lab

1. Dissecting UK drought tolerance in Paragon x Garcia
2. Quantifying agronomic impact of WGIN target genes using the Paragon NIL library

4. A chromosome segment substitution library for Avalon x Cadenza

1. UK drought tolerance in Paragon x Garcia

- Single-rep 1m field trial 2014-15
- Weather data



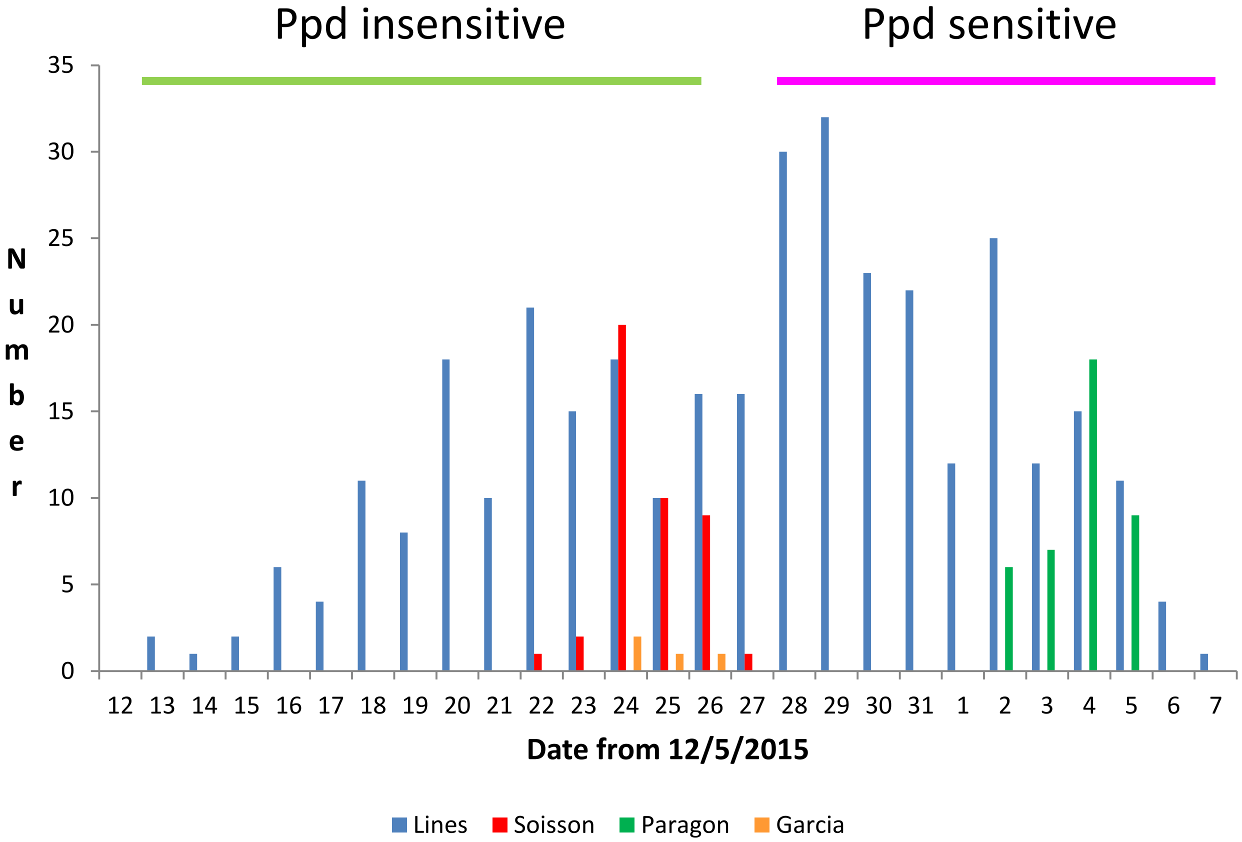
LSP = Seedling emergence to first node detectable
SEP = First node detectable to anthesis
GFP = Anthesis to physiological maturity

1. UK drought tolerance in Paragon x Garcia

- Single-rep 1m field trial 2014-15
- Weather data
- F6 scored for DTEM, Height, Yield and TGWT*
- QTL mapping, using Single-Trait Linkage Analysis (Single Environment) in Genstat 16

Drought Tolerance

DTEM



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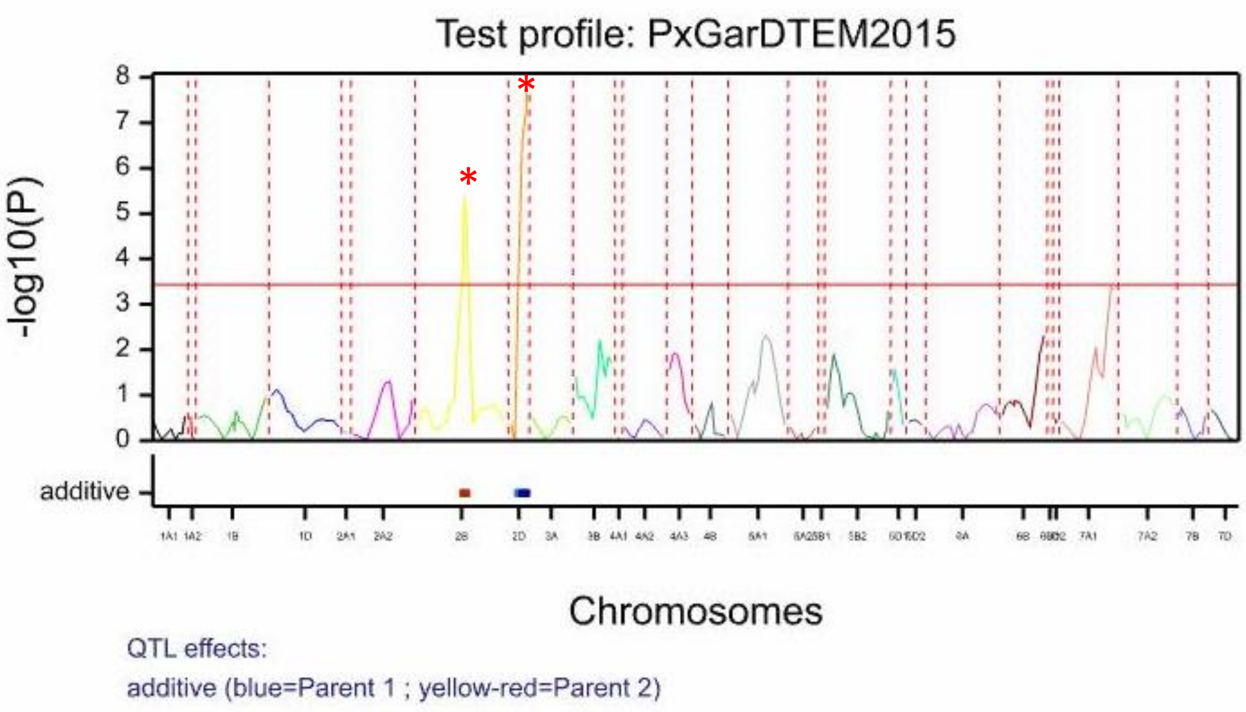
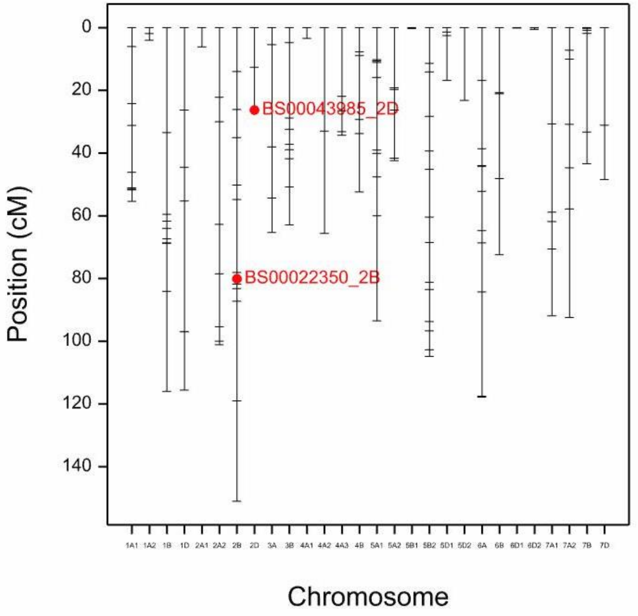
Drought Tolerance

DTEM



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



QTL effects

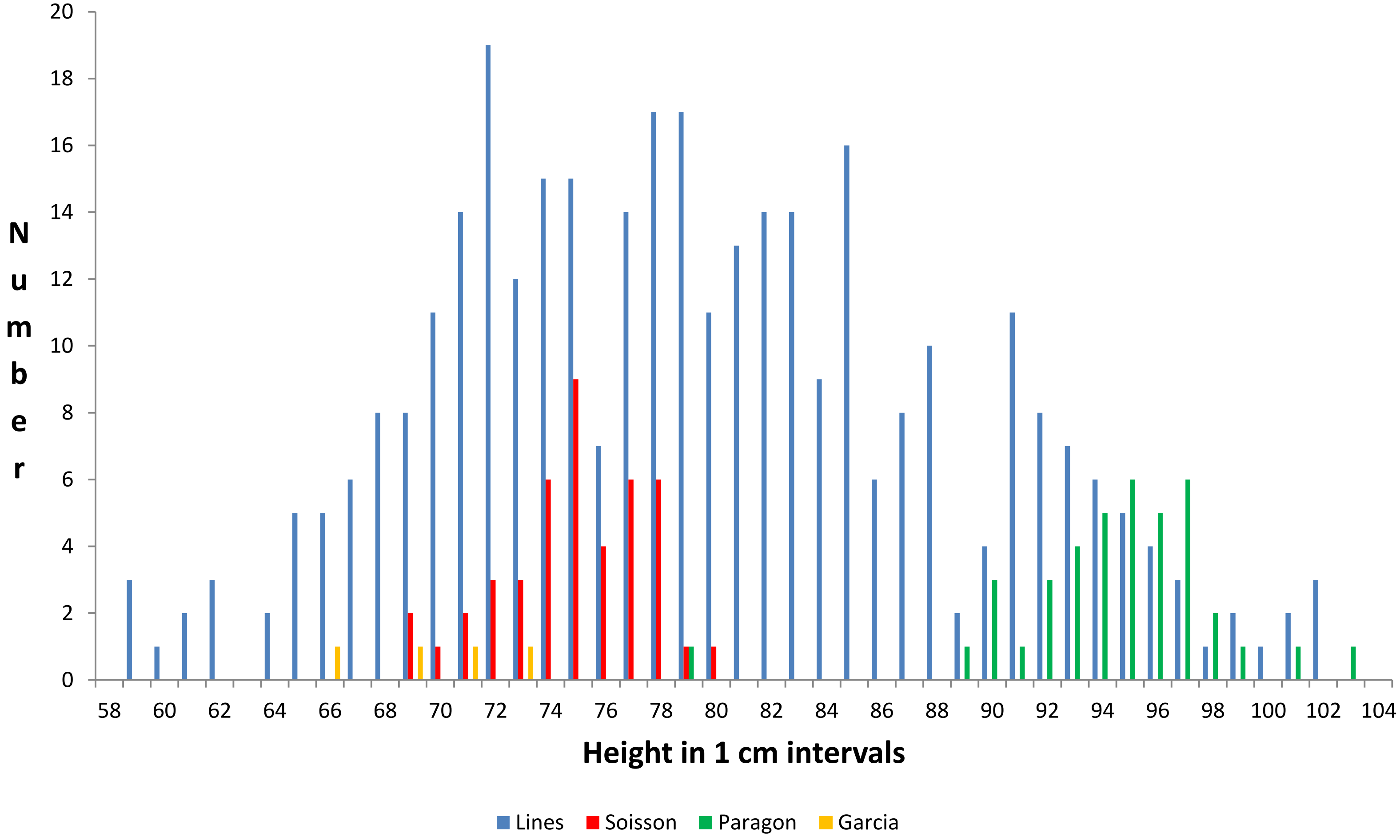
Locus no.	Locus name	%Expl. Var.	Add. eff.	High value allele	s.e.
80	BS00022350_2B	5.652	1.312	Garcia	0.285
98	BS00043985_2D	8.463	1.605	Paragon	0.286

2B Homologue HvCEN?

2D PpdD1?

Drought Tolerance

Height



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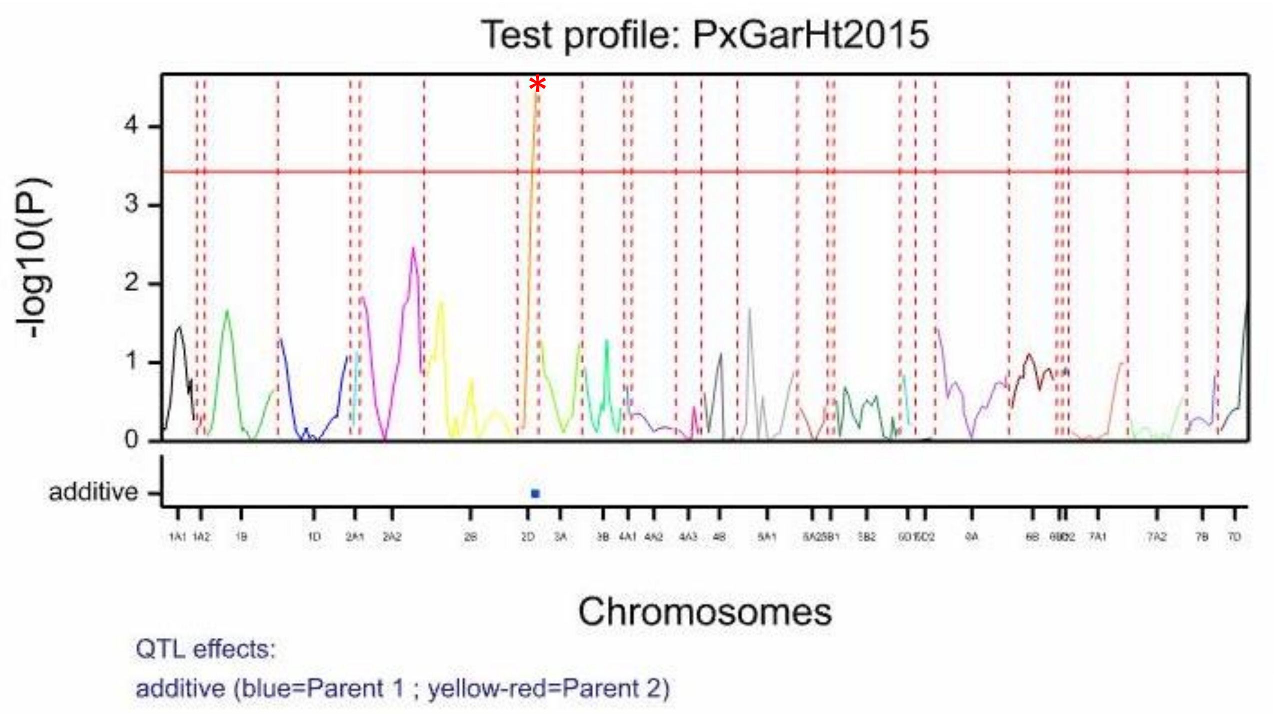
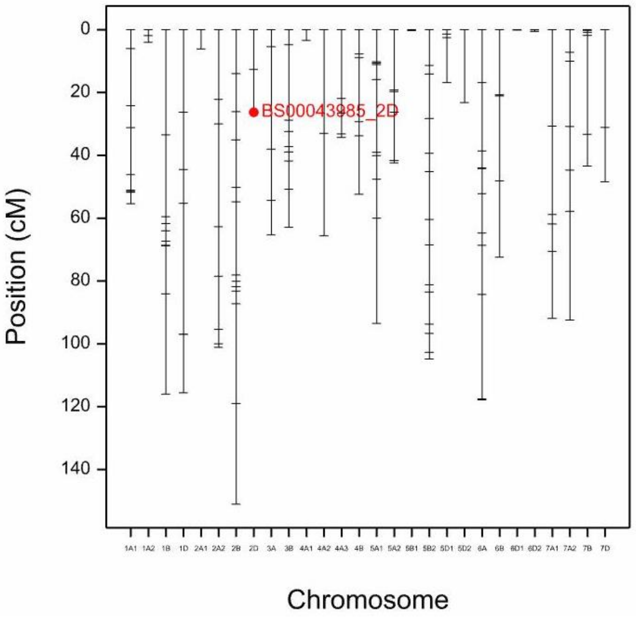
Drought Tolerance

Height





QTL effects

Locus no.	Locus name	%Expl. Var.	Add. eff.	High value allele	s.e.
98	BS00043985_2D*	5.057	2.049	Paragon	0.496

Close to known Height QTL on 2D

*QTL for DTEM and Ht

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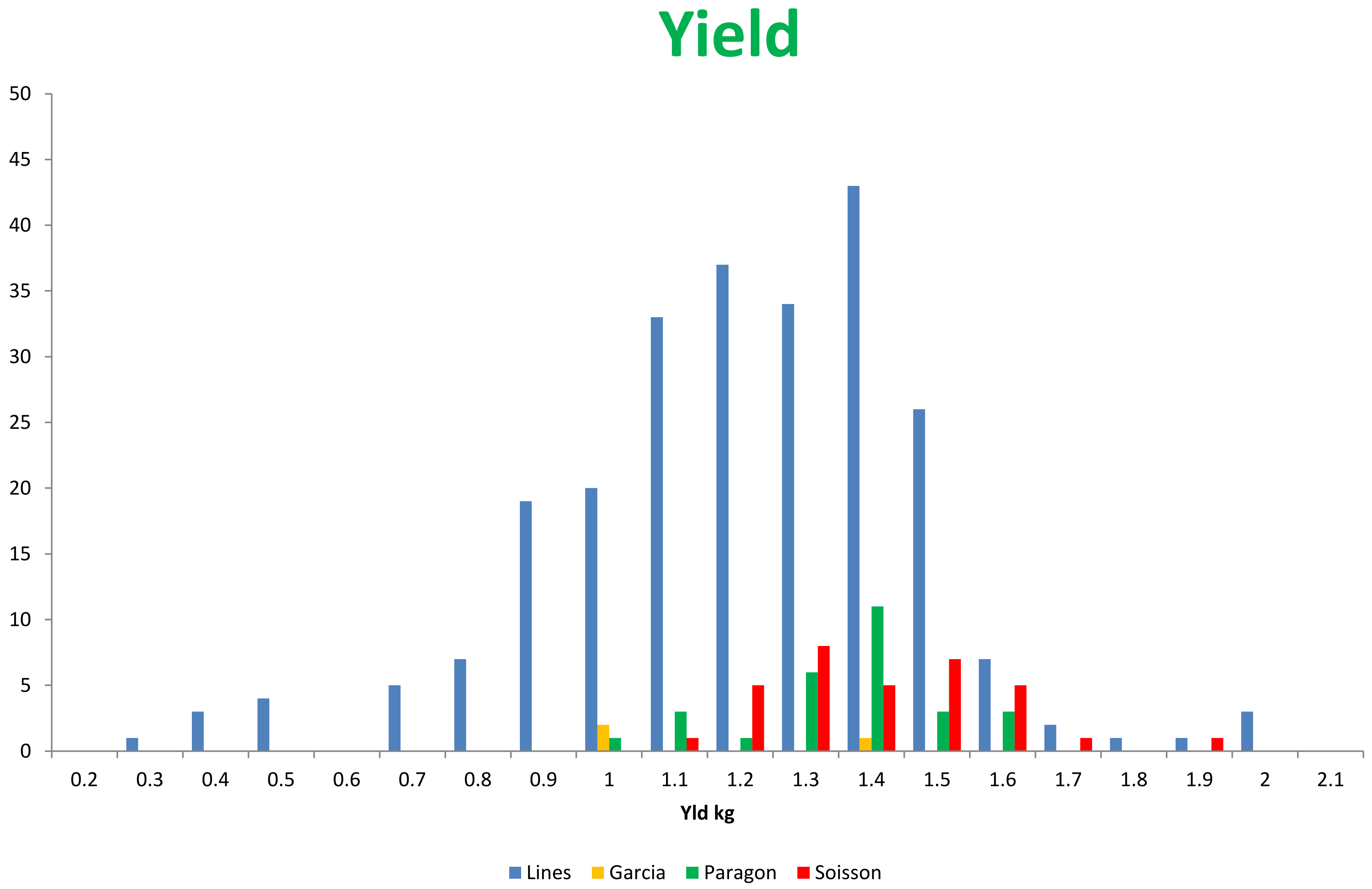
Drought Tolerance



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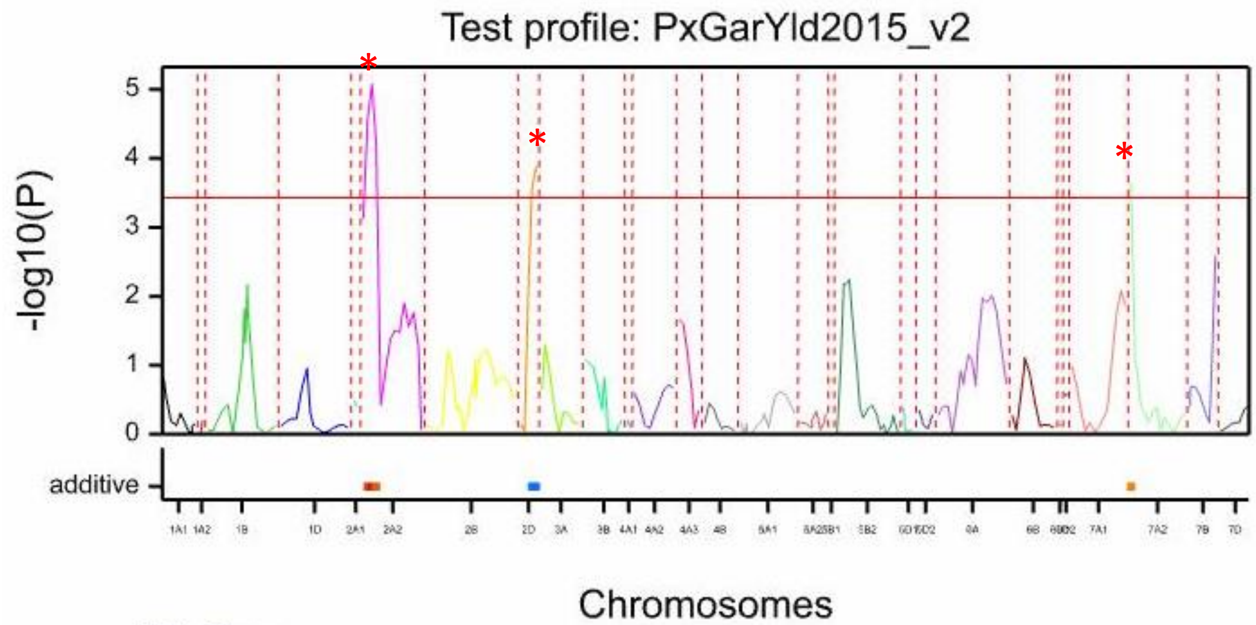
Drought Tolerance

Yield



QTL effects:
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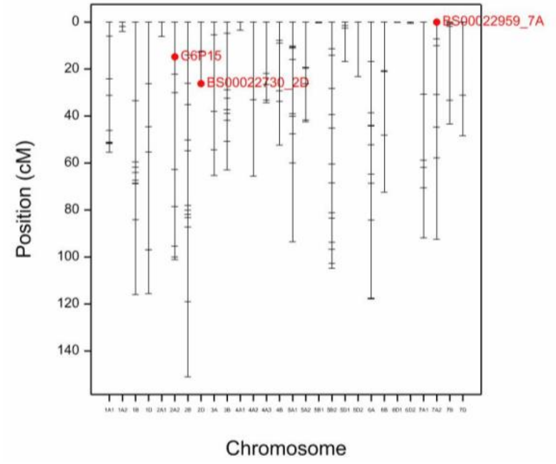


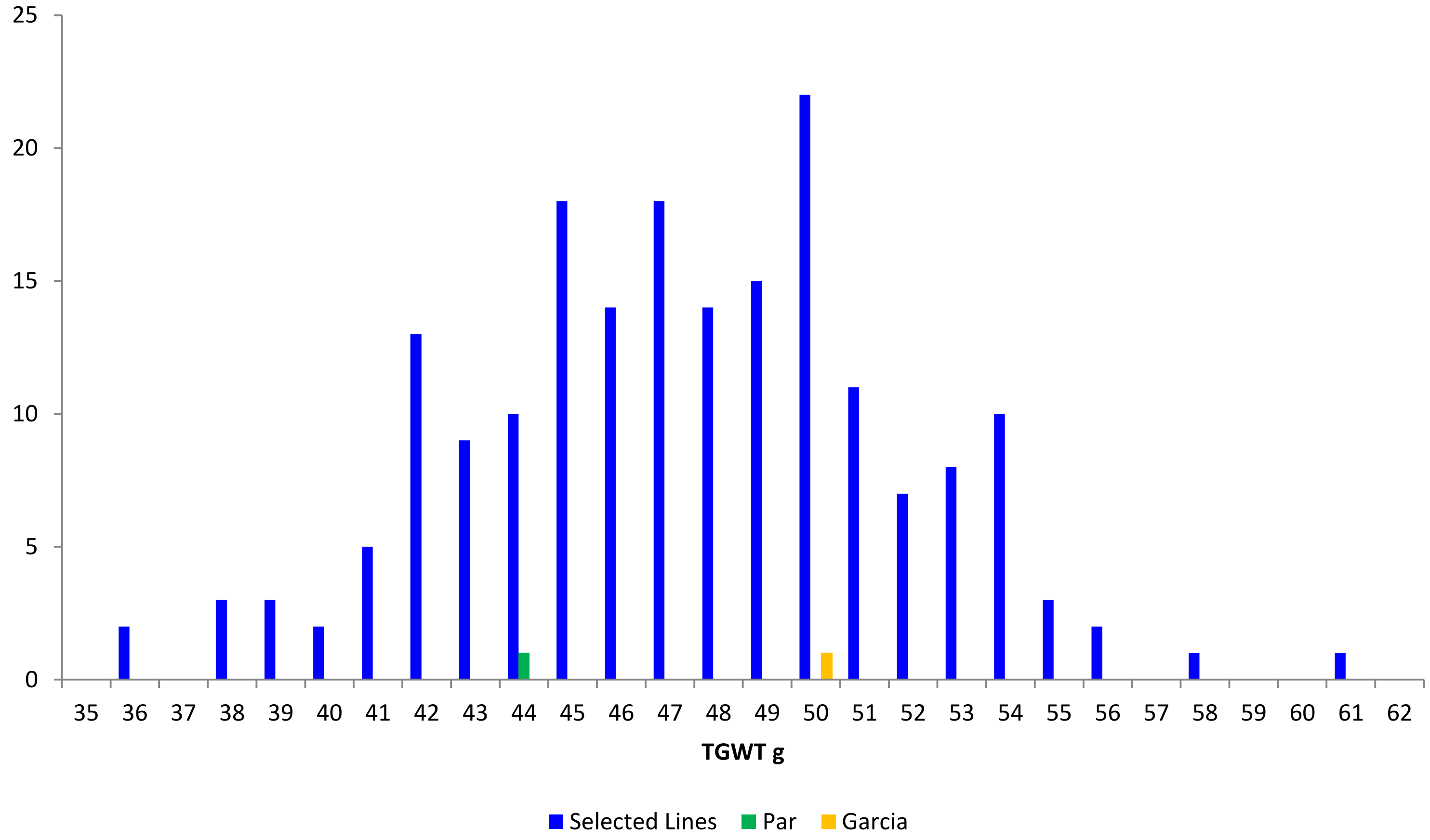
QTL effects

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?

Drought Tolerance

TGWT*



* Lines selected for drought trial only - Ppd-sensitive

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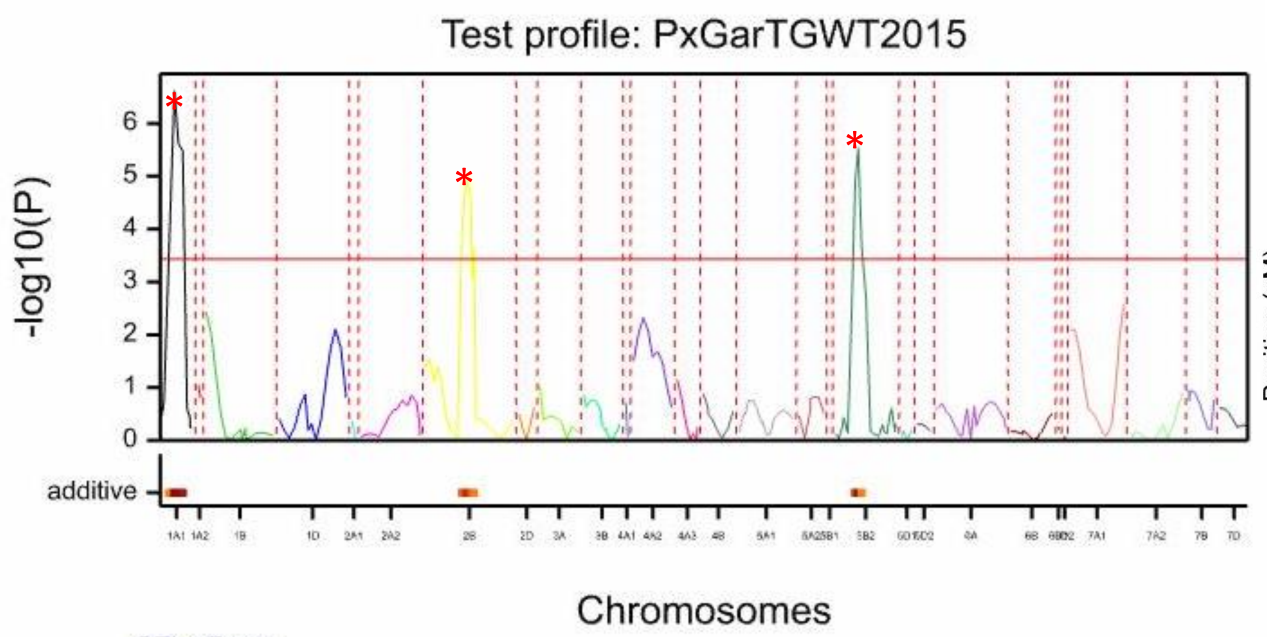
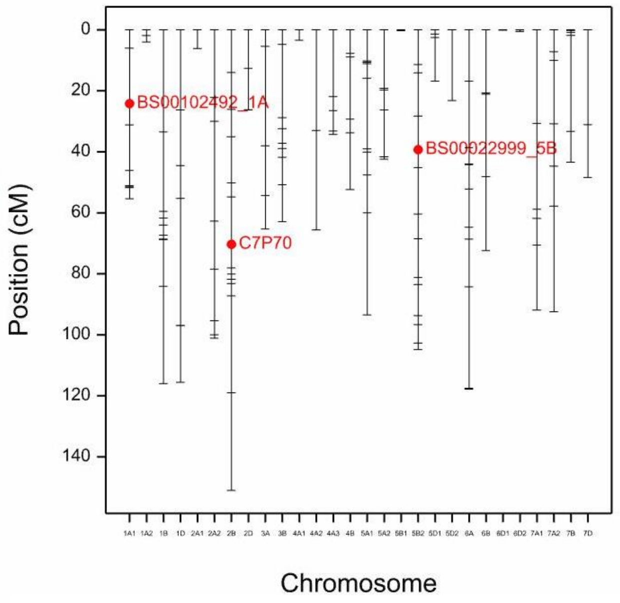
Drought Tolerance

TGWT*



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



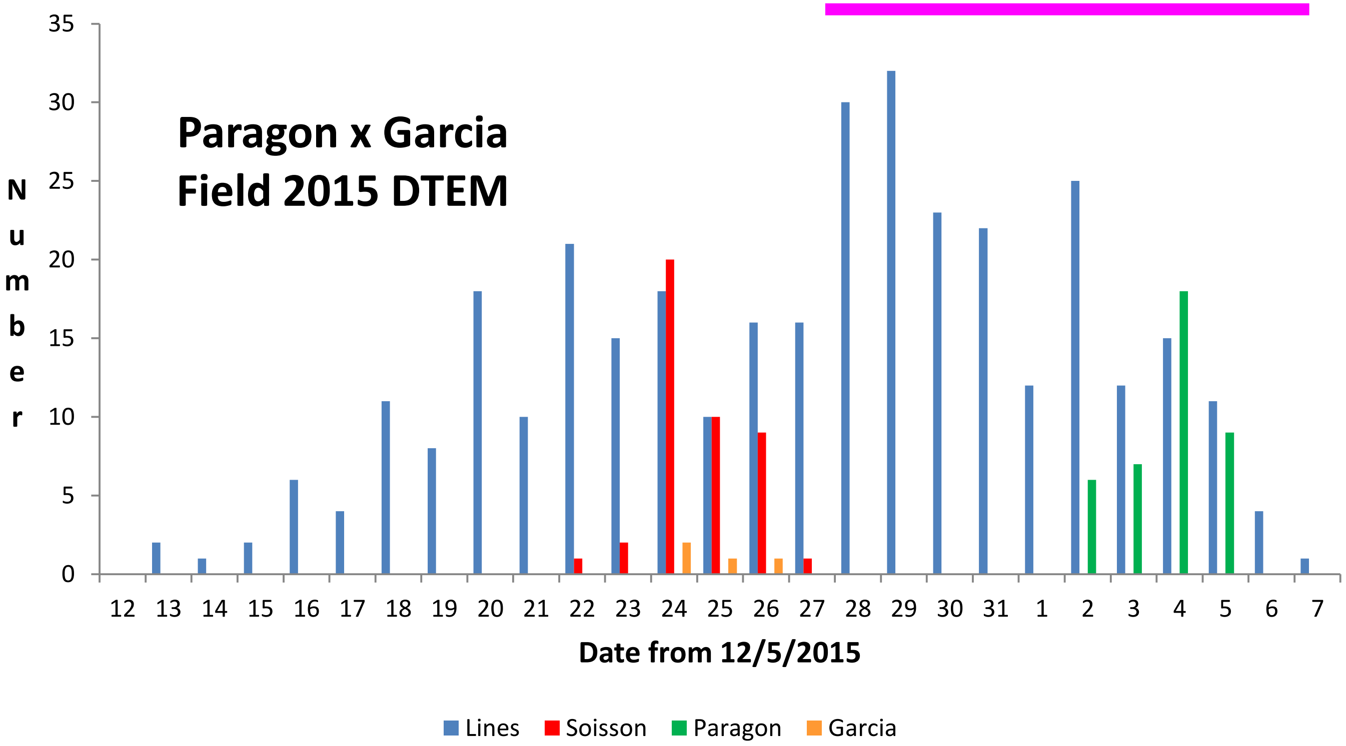
QTL effects

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?

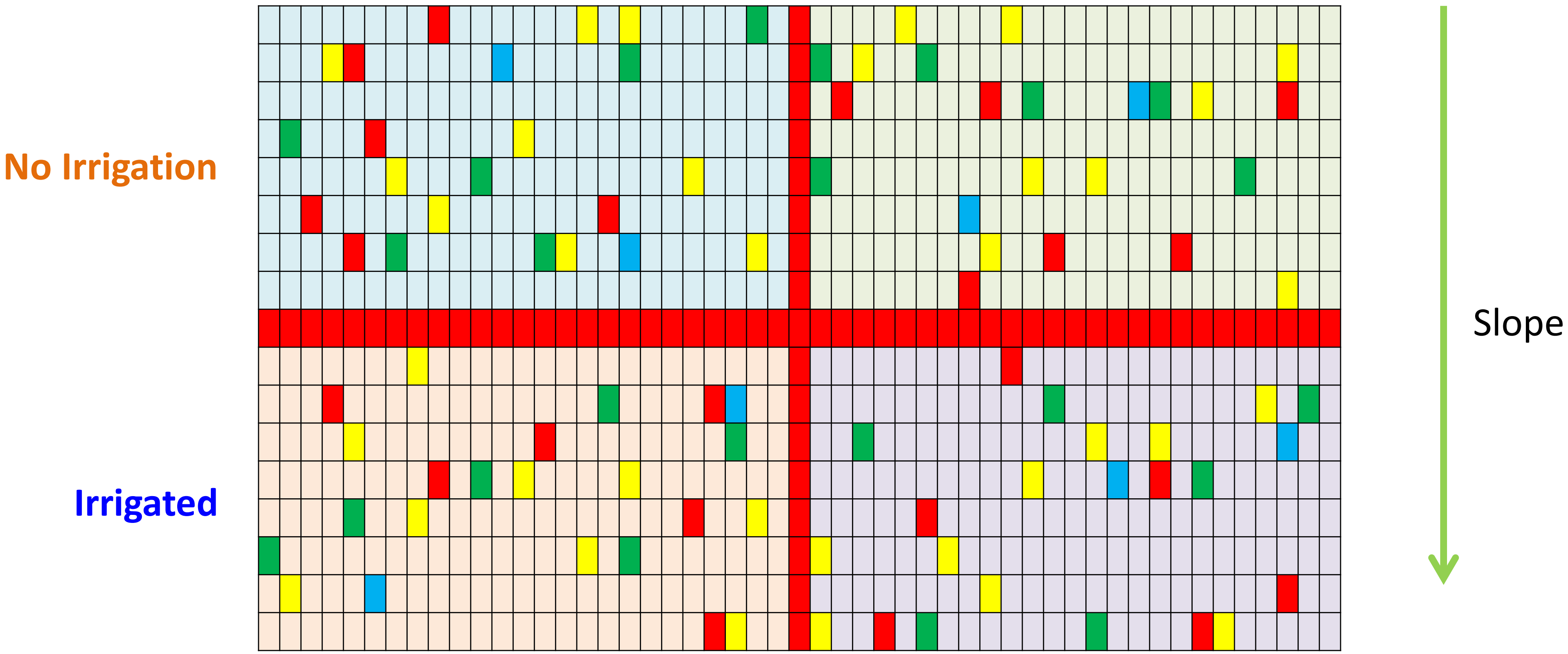
Drought Tolerance

- Drought Trial drilled with selected P x Gar lines (Ppd-sensitive)



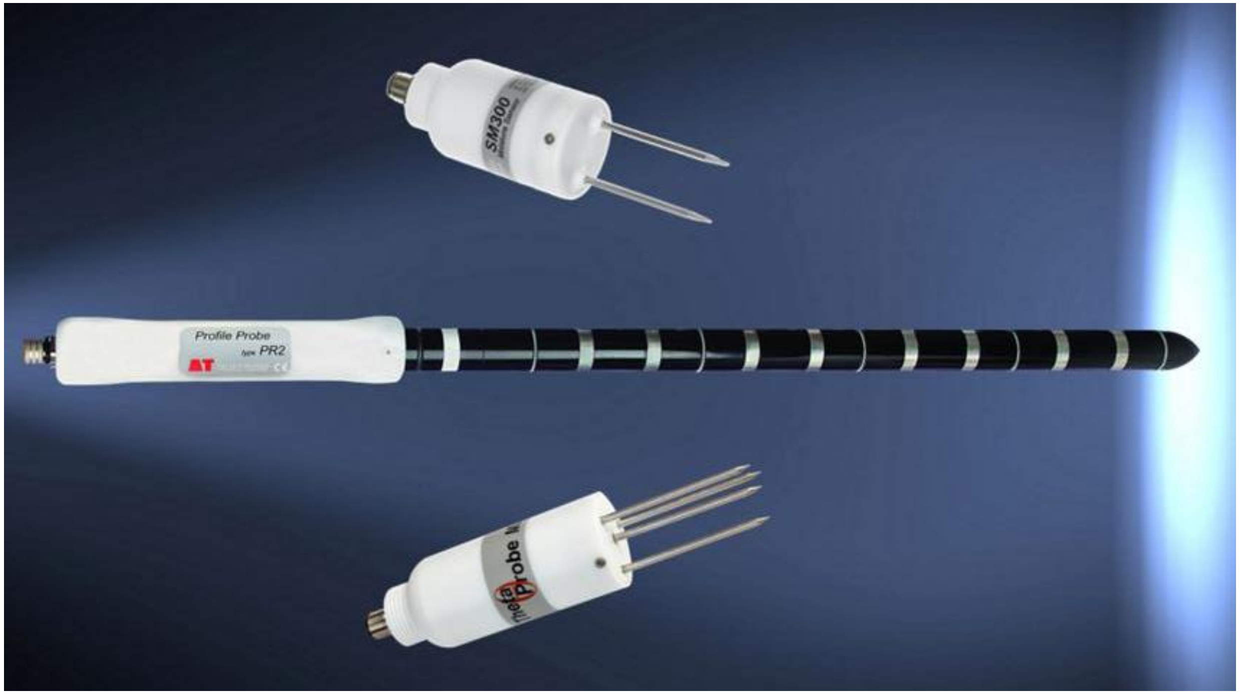
Drought Tolerance

- 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**



Drought Tolerance

- Investigating methods of measuring, soil temperature, soil water content and water potential
- Collaborating with John Foulkes (Uni Nott.)
- Considering monitoring equipment from **DeltaT**



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

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

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

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

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

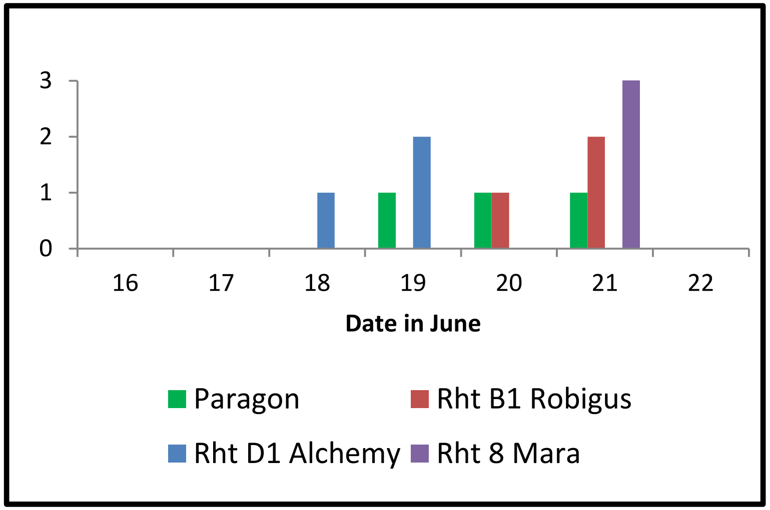
Paragon NIL library (subset)

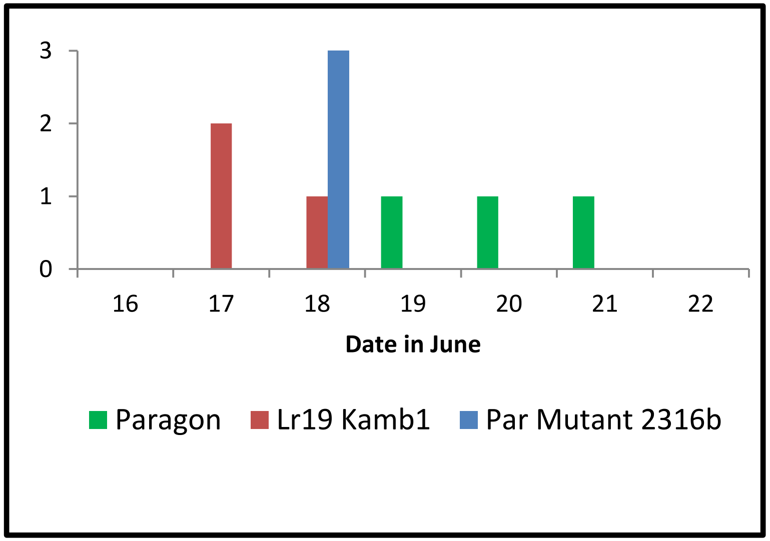
Paragon	Par Library	
Lr19 Kamb1	Par Library 2-15	Alien introgression*
Par Mutant 2316b	Par Library 20-9	Staygreen
Ppd 1x Early	Par Library 15-12	DTEM
Ppd 2x Early	Par library 16-7	DTEM
Ppd 3x Early	Par library 16-15	DTEM
Ppd KO 3x	Par Library 19-1	DTEM
Rht 8 Mara	H14 Nor N Med Irr-9	Height
Rht B1 Robigus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

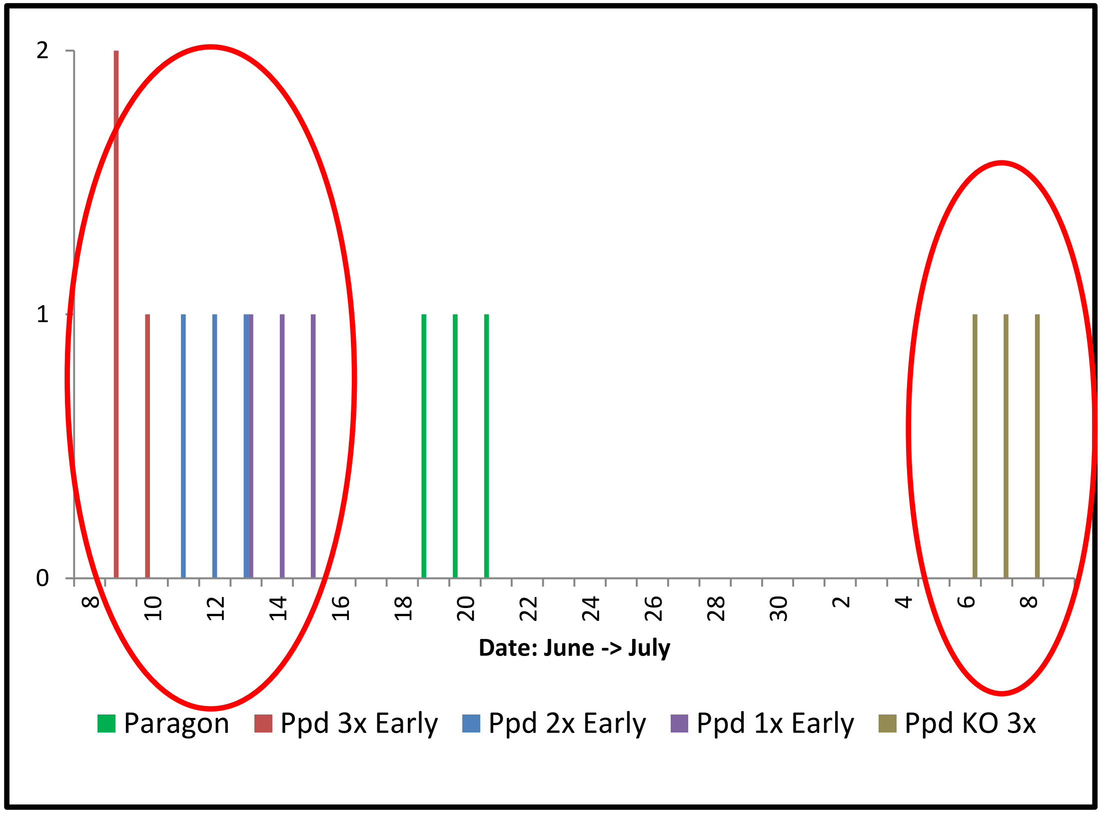
*Leaf rust resistance gene on 7DL, derived from *Agropyron elongatum*

Paragon NIL library (subset)

- DTEM







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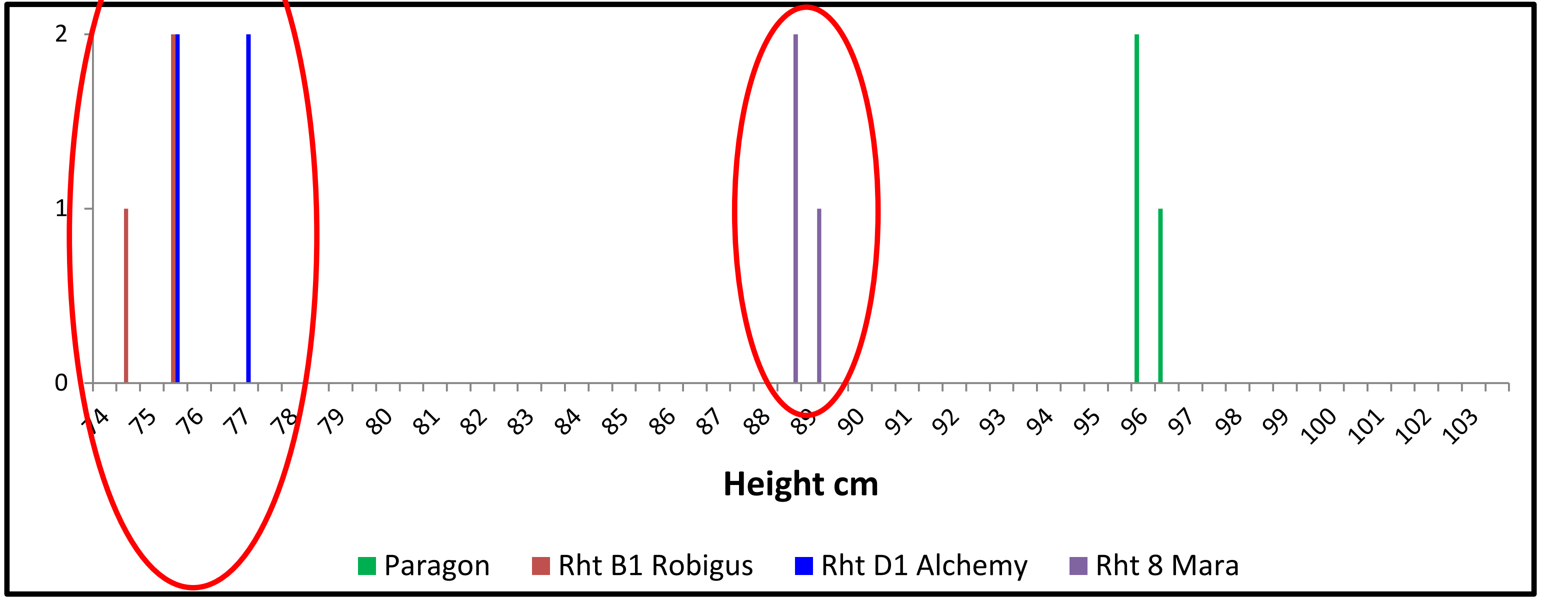
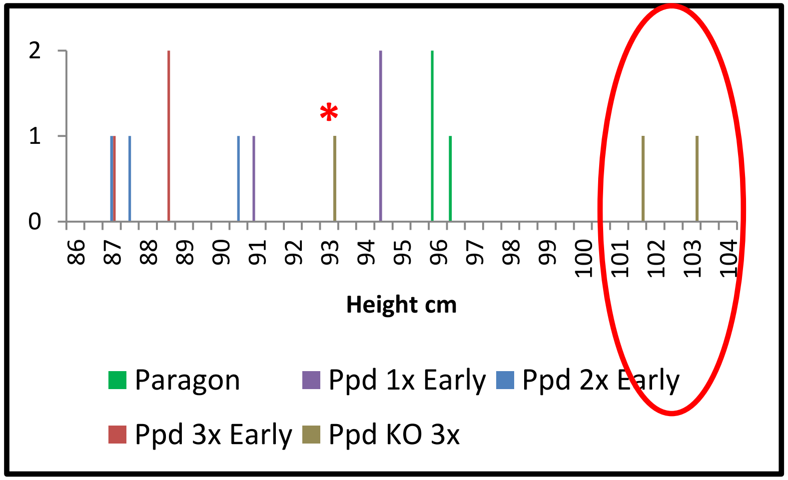
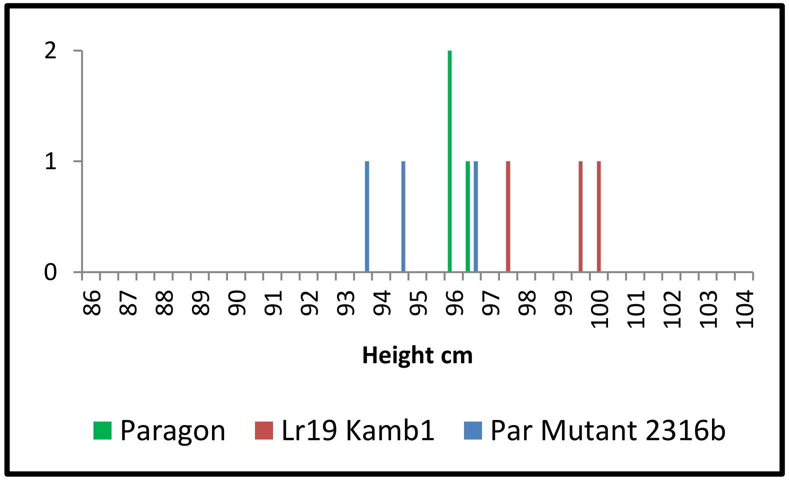
Paragon NIL library (subset)

- **Height**

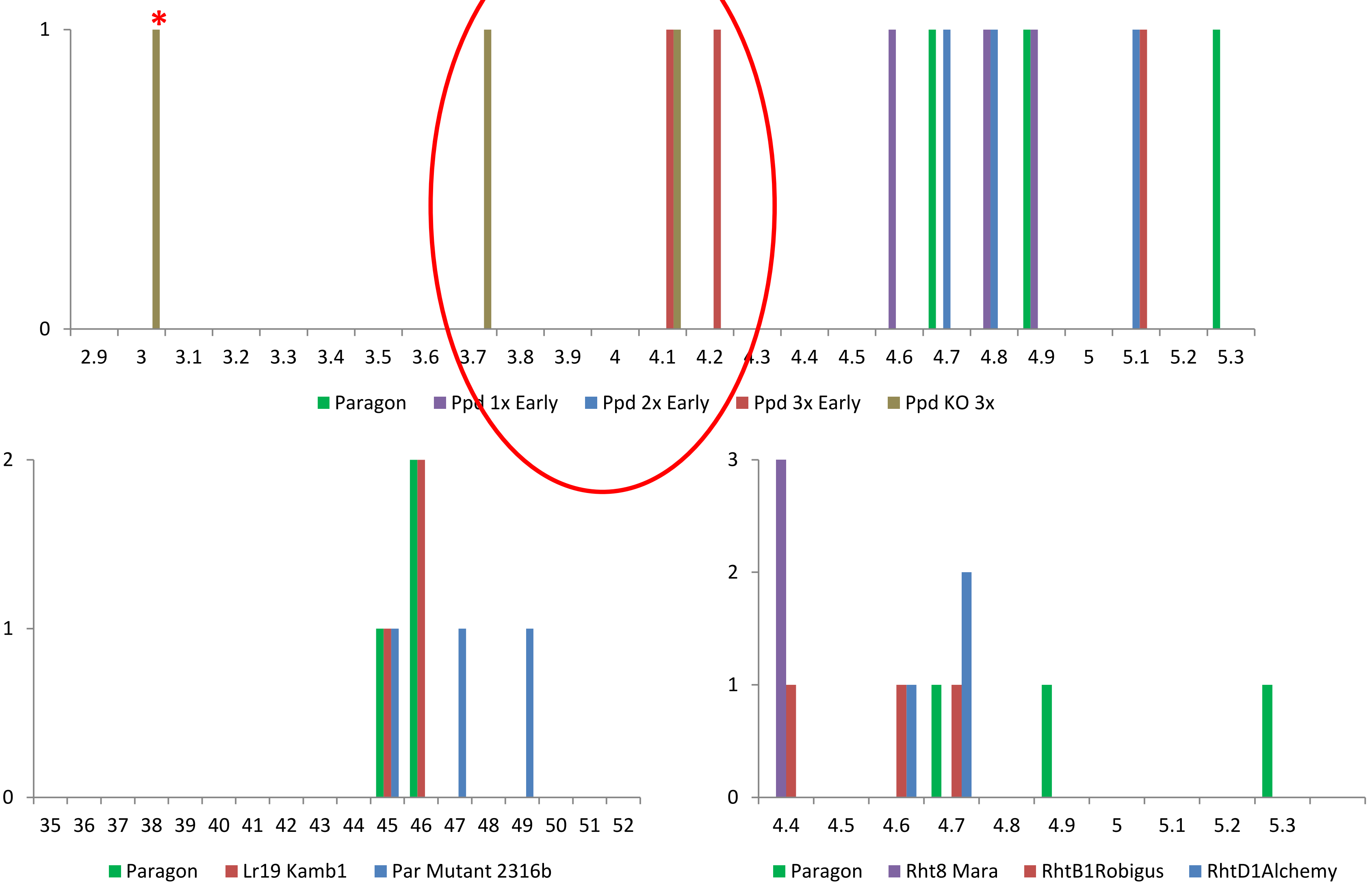






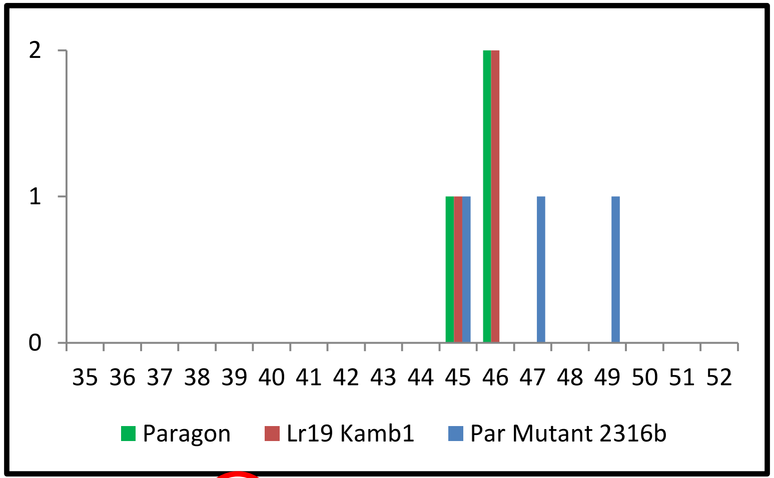
Paragon NIL library (subset)

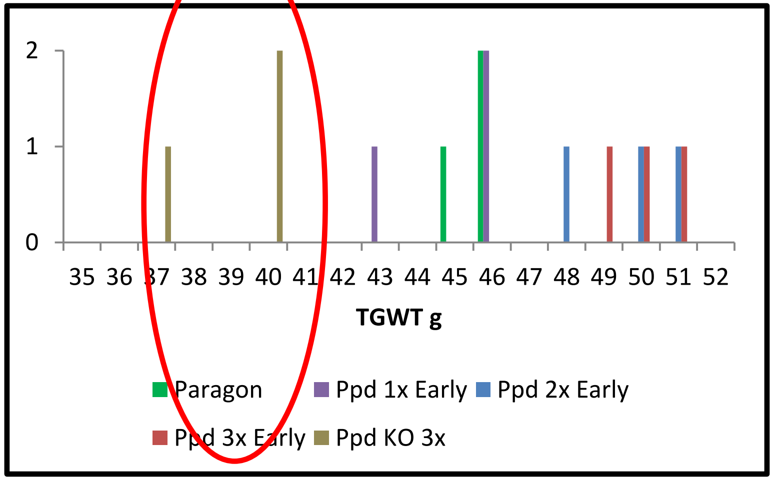
- **Yield**

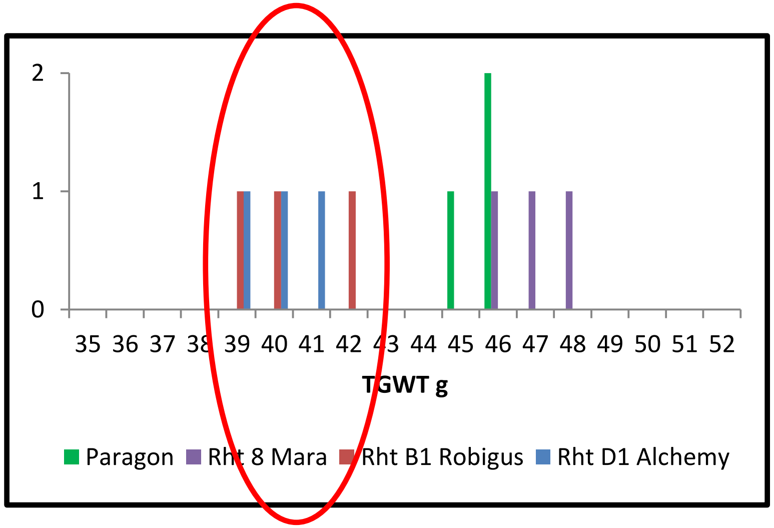


Paragon NIL library (subset)

- **TGWT**







Paragon NIL library (subset)

- Paragon Library subset yield trial being repeated
- Also included in Drought Trial
- Eight lines will be drilled under Phenospex
    - Two randomised blocks of 16 +/- additional nitrogen

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

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

- The BC3 NILs, derived from the A x C DH population, carry selected genetic foreground in the QTL regions
- In addition each line carries ~12.5% random chromosomal regions.

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CSSL for Avalon x Cadenza

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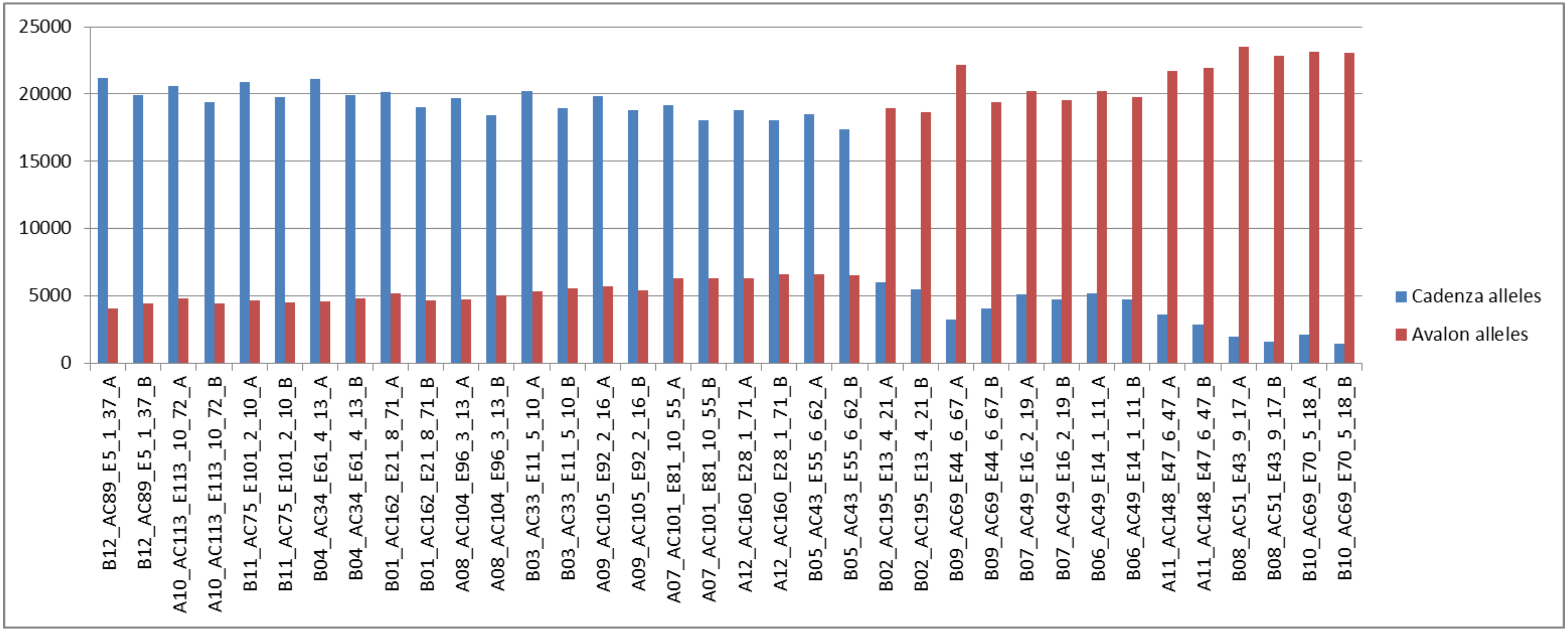


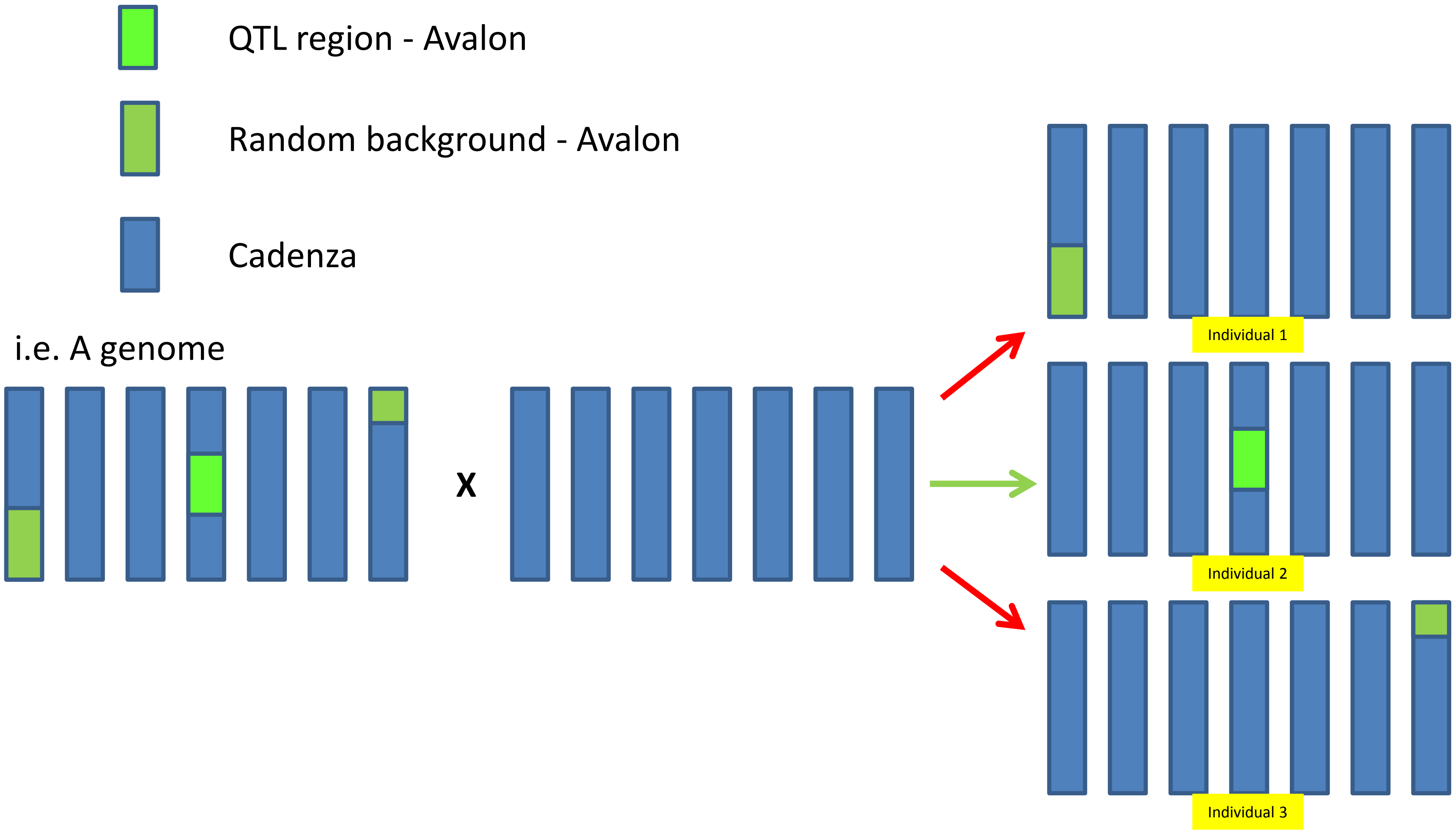
18 NILs genotyped on the 820K array

CSSL for Avalon x Cadenza

- The BC3 NILs, derived from the A x C DH population, carry selected genetic foreground in the QTL regions
- In addition each line carries ~12.5% random chromosomal regions.
- Anticipated that in 552 NILs* every locus of Cadenza will be represented in Avalon, and *vice versa*
- Can we ‘tile’ the whole genome to make recombinant substitution lines for the whole genome in both Avalon and Cadenza backgrounds?

*250 BC_2 NILs in Avalon background, 302 BC_2 NILs in Cadenza background

CSSL for Avalon x Cadenza



🟩 QTL region - Avalon

🟩 Random background - Avalon

🟦 Cadenza

i.e. A genome

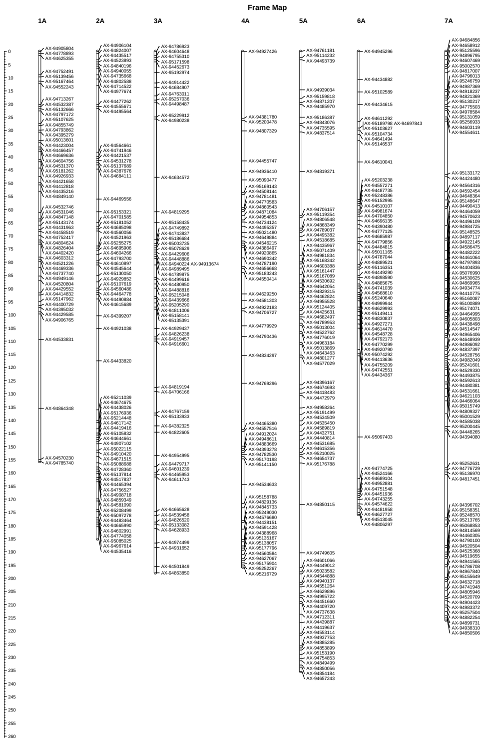
CSSL for Avalon x Cadenza

- Full A x C Map (18942 markers) from Bristol*
- Frame A x C Map (1286 markers) from Bristol

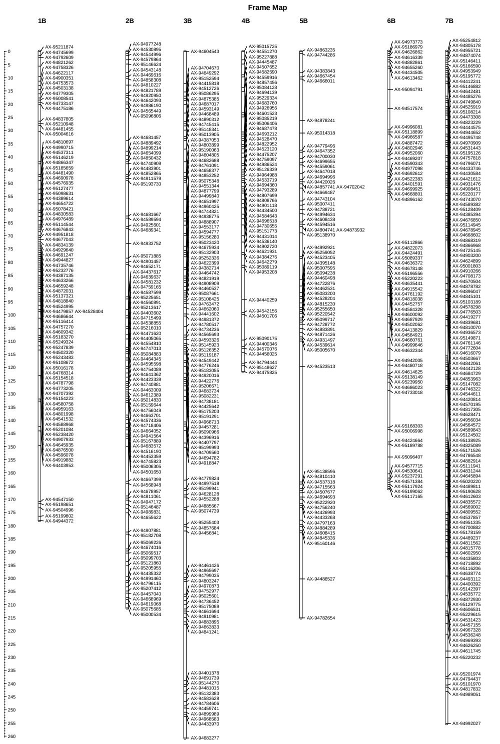
*“High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool” Wingfield et al 2015. **PBJ**

CSSL for Avalon x Cadenza

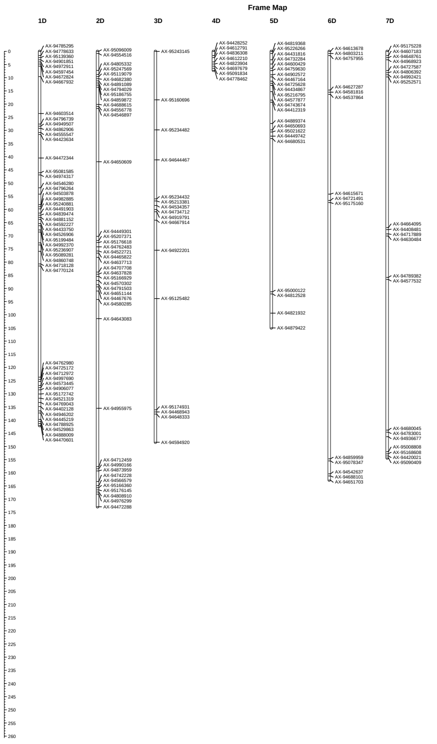
A Genome



B Genome



D Genome



Frame AxC Map (1286 markers)

CSSL for Avalon x Cadenza

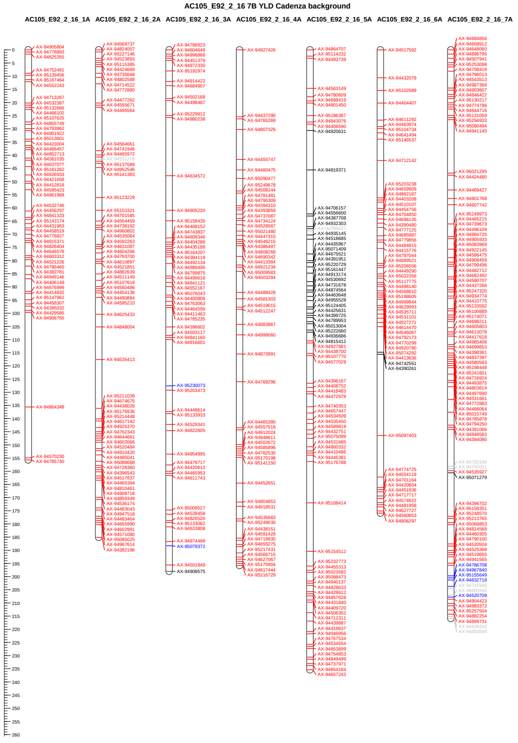
- Full AxC Map (18,942 markers) from Bristol*
- Frame AxC Map (1,286 markers) from Bristol

- 820K Axiom data for 18 NILs
- Maps based on Frame AxC map (1,260 markers)

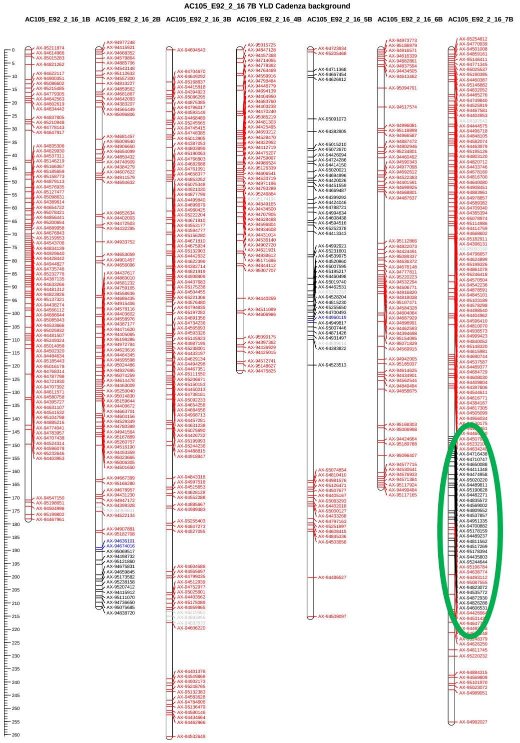
*"High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool" Wingfield et al 2015. **PBJ**

CSSL for Avalon x Cadenza

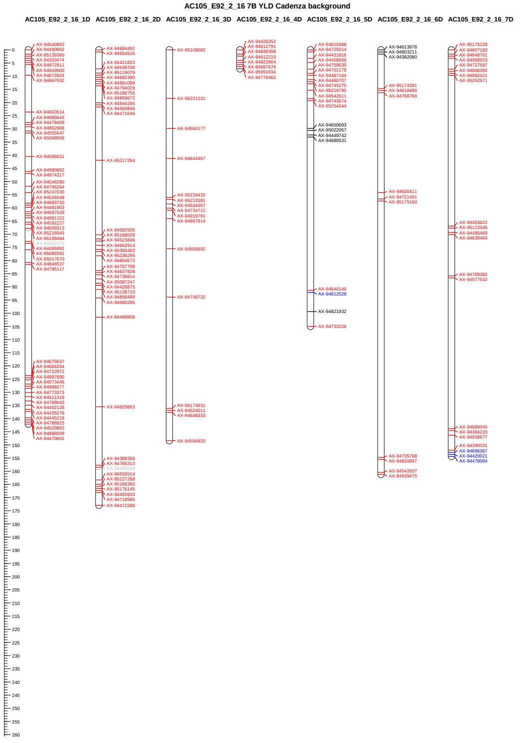
A Genome



B Genome



D Genome



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

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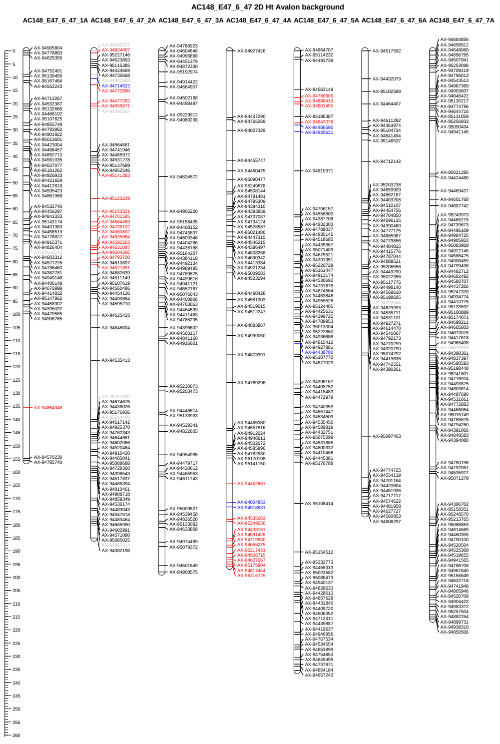
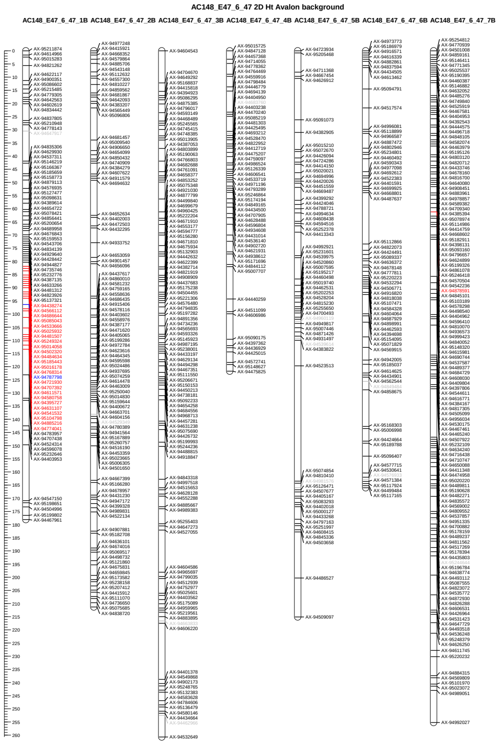
CSSL for Avalon x Cadenza

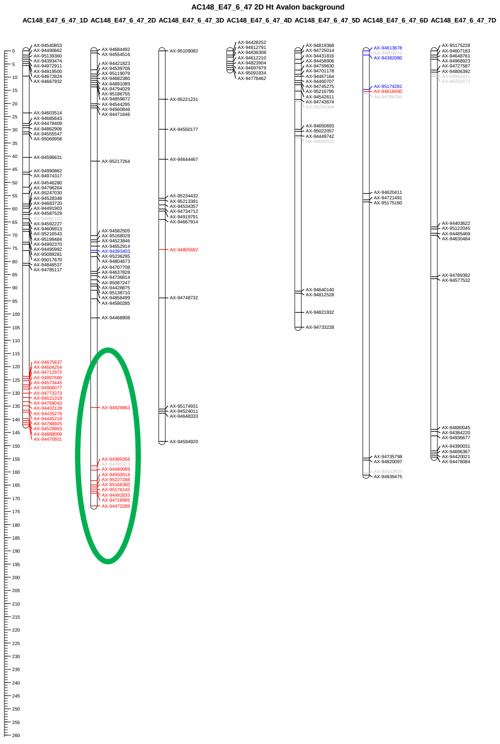
A Genome



B Genome



D Genome



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

CSSL for Avalon x Cadenza

- Chromosomal coverage in the NILs genotyped on the 820K…
- Is there sufficient coverage without further genotyping?
- Unfortunately not!

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

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CSSL for Avalon x Cadenza

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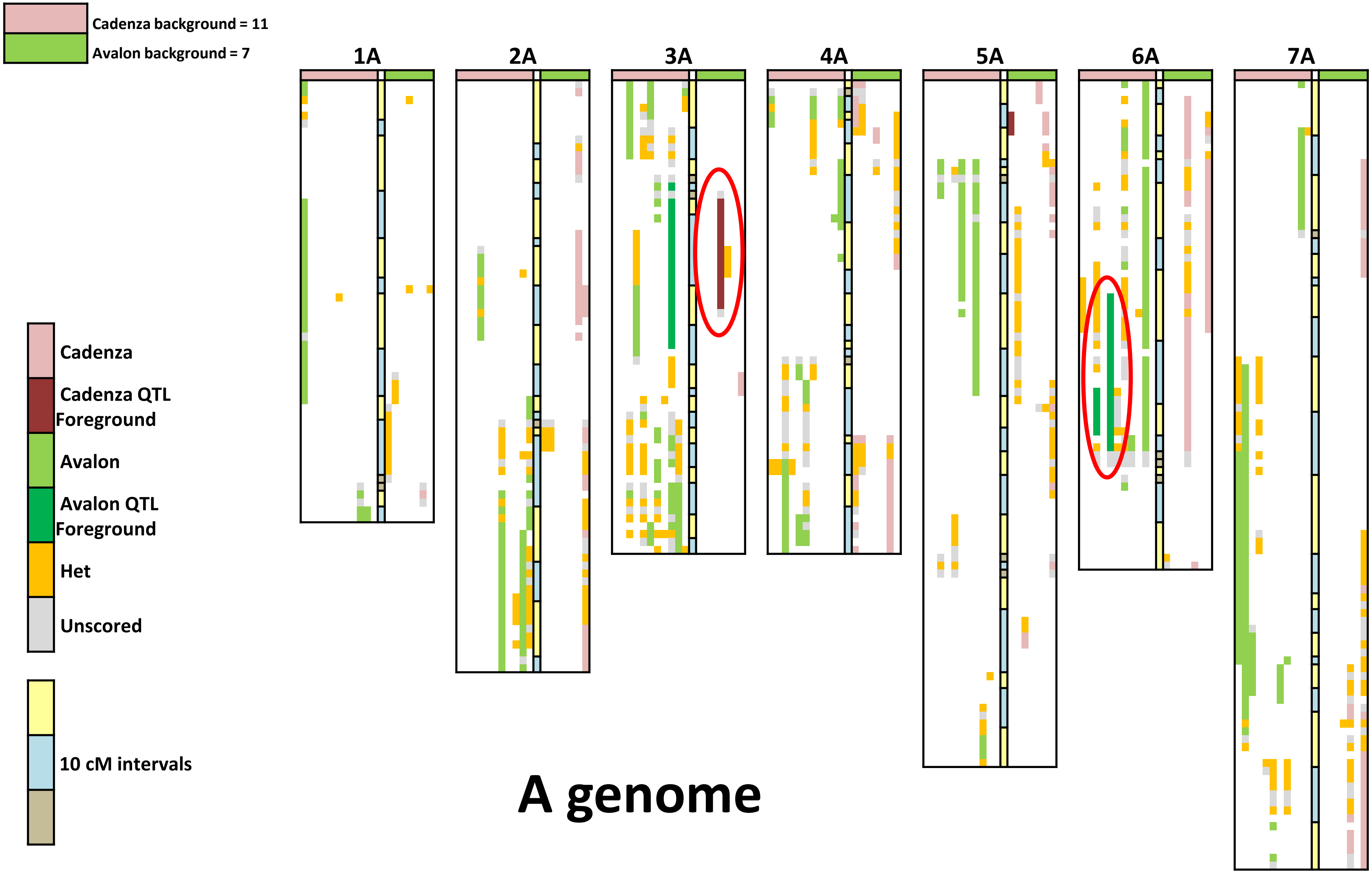

A genome

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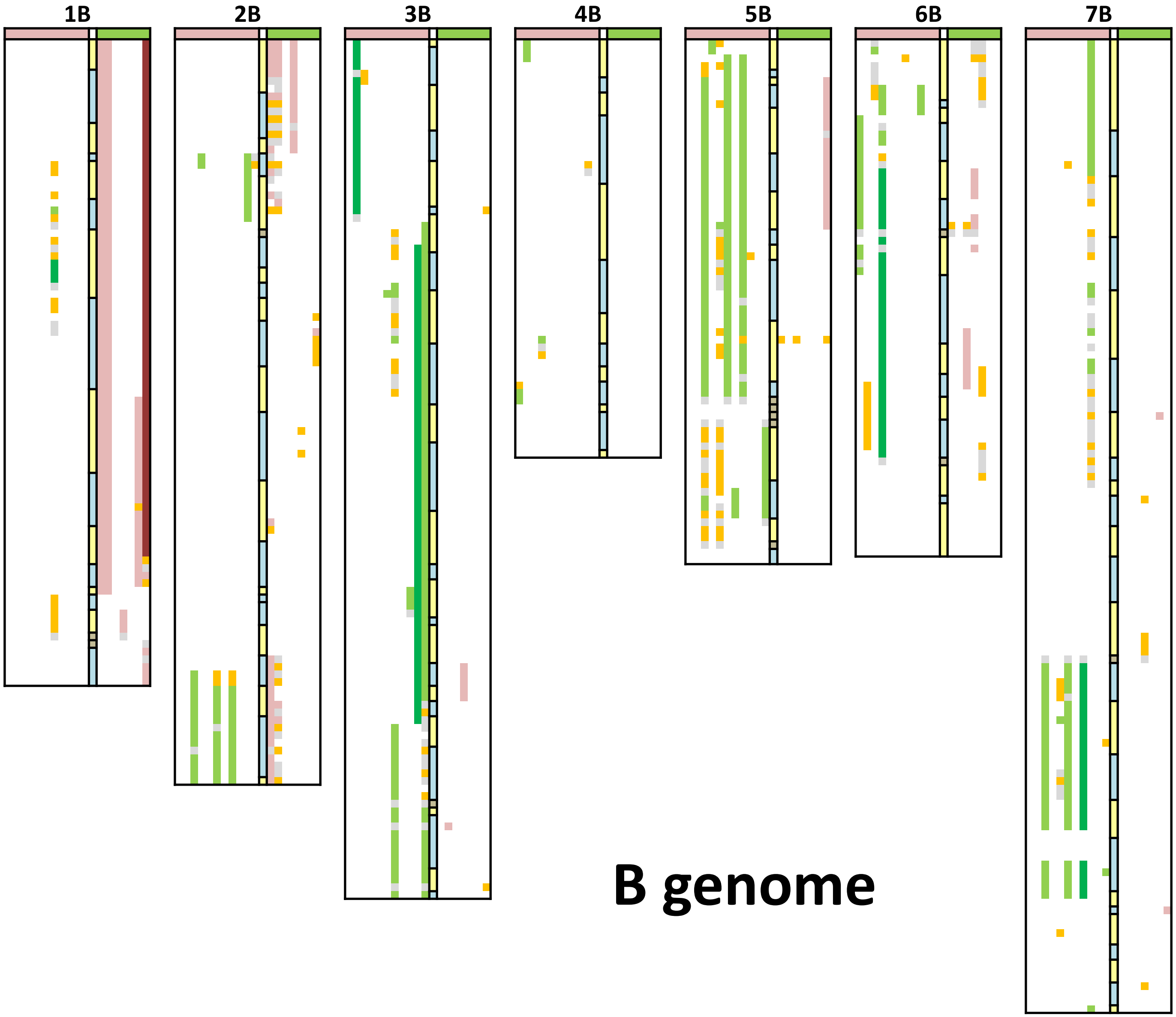
CSSL for Avalon x Cadenza

🟥 Cadenza background
🟩 Avalon background



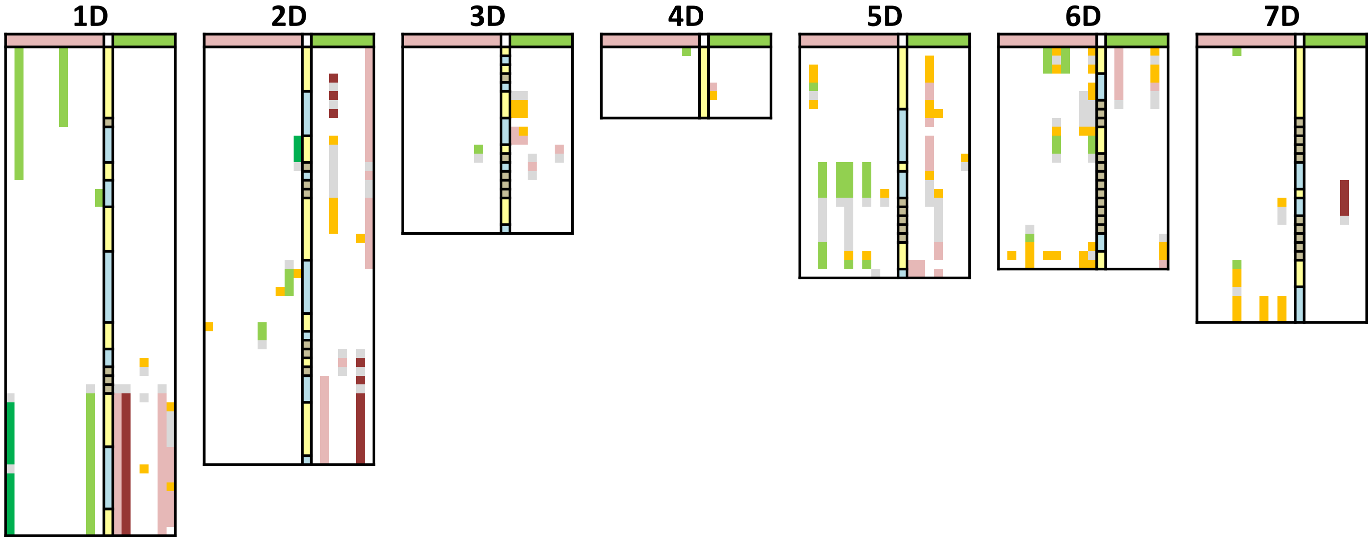
🟥 Cadenza
🟥 Cadenza QTL Foreground
🟩 Avalon
🟩 Avalon QTL Foreground
🟧 Het
⬜ Unscored

🟨🟦🟫 10 cM intervals

B genome

CSSL for Avalon x Cadenza

🟥 Cadenza background
🟩 Avalon background



🟥 Cadenza
🟥 Cadenza QTL Foreground
🟩 Avalon
🟩 Avalon QTL Foreground
🟧 Het
⬜ Unscored

🟨 🟦 🟫 10 cM intervals

D genome

CSSL for Avalon x Cadenza

- Genotyping of 94 lines on 35K Axiom array
- Need markers in 820K array also in 35K for maps
- 47 Avalon+b allele and 47 Cadenza+a allele lines
- Representing all the AxC QTLs (EM, Ht, YLD)

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

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

CSSL for Avalon x Cadenza

- Lines growing in glasshouse – have been vernalized
- Will be backcrossed to Avalon or Cadenza
- F1 ready early spring

- DNA not yet ready but not limiting factor

WGIN3 Management Meeting

28th October 2015

Screening germplasm for resilience to aphids (WP2.3)

Lesley Smart

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BBSRC
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

ROTHAMSTED RESEARCH

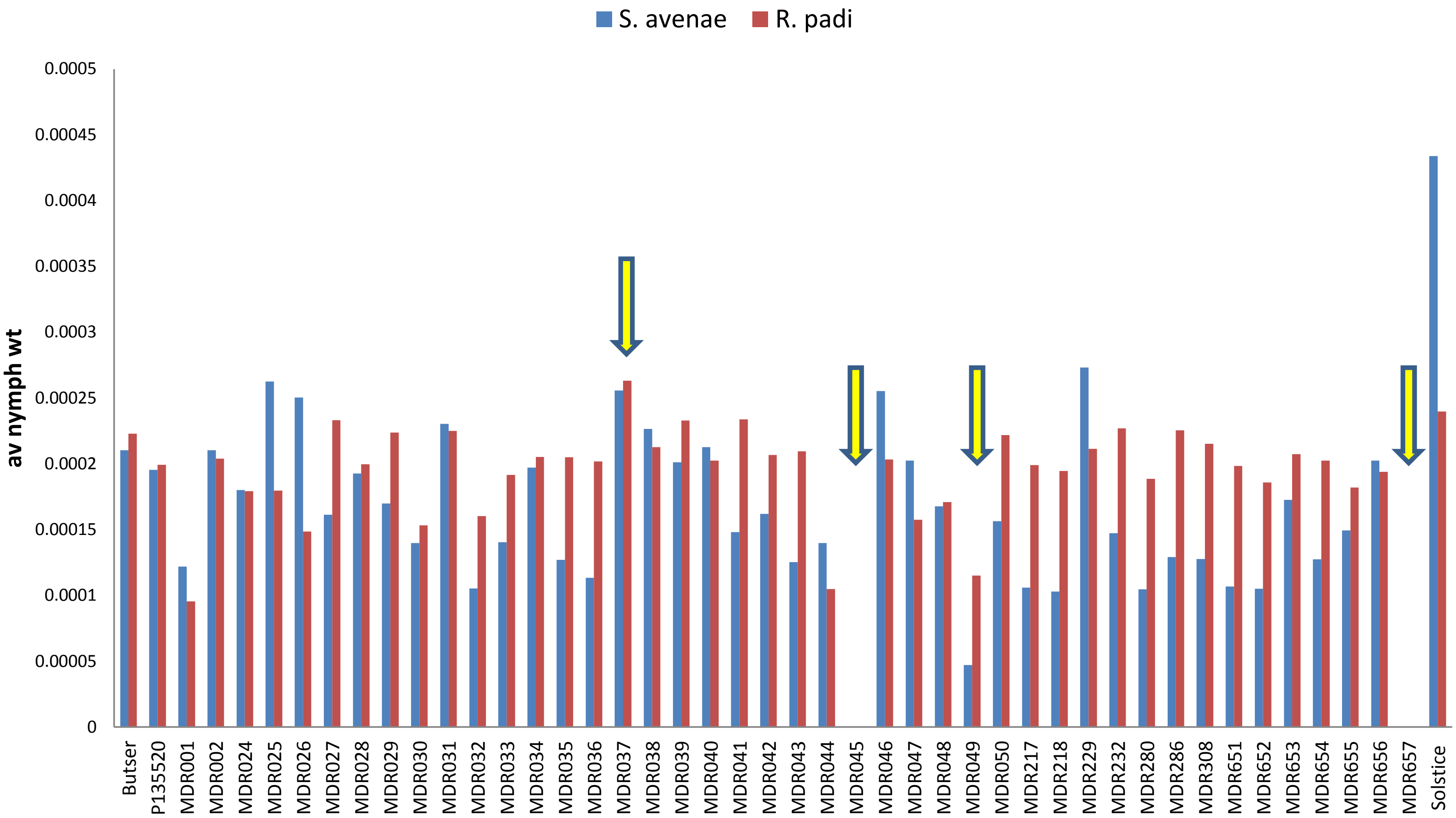
- Focus on *Triticum monococcum* lines as these provided the most promising leads for partial resistance to cereal aphids from work in WISP
  - Fewer nymphs were produced compared to hexaploid varieties
  - Reduced weight gain by the nymphs over time – disrupted feeding patterns
  - Slower development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

Wheat Genetic Improvement Network

BBSRC bioscience for the future

- **Focus on *Triticum monococcum* lines**

Nymph weight on *Triticum monococcum* (MDR) lines in aphid screening assay (WISP)



WISP

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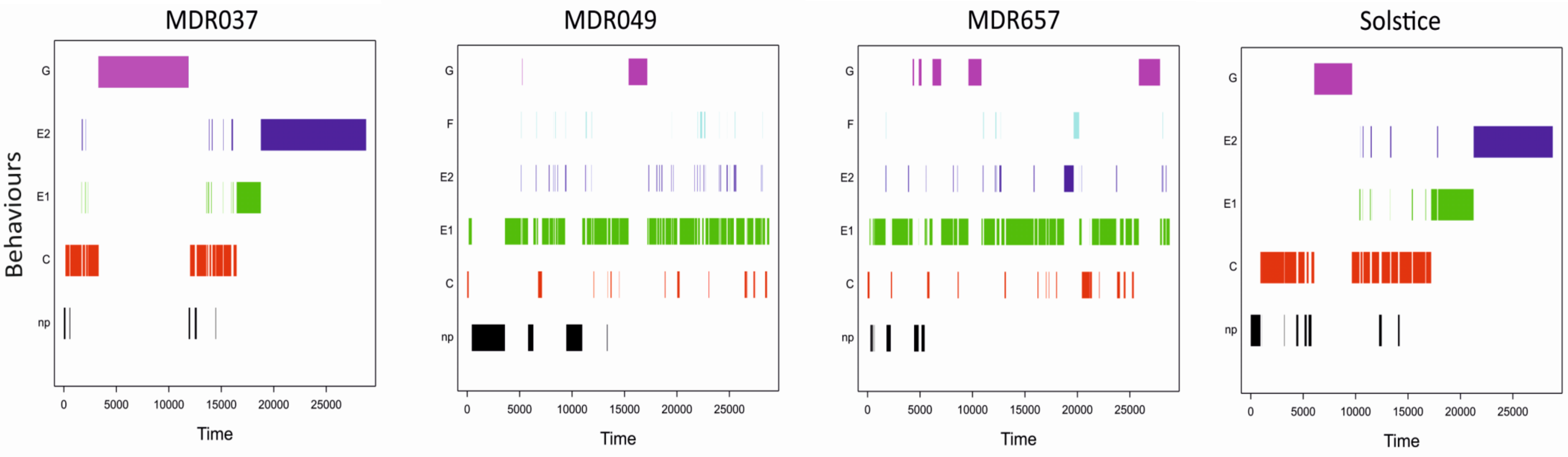
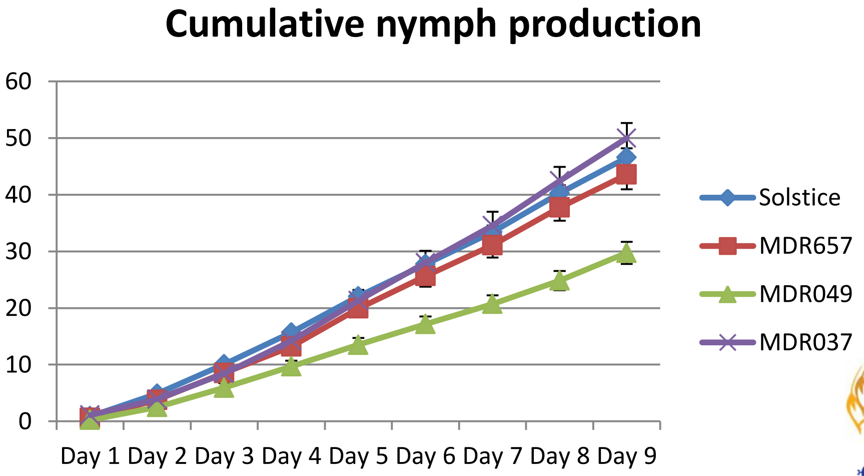
Reduced feeding by *R. padi in* EPG bioassays in WISP

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

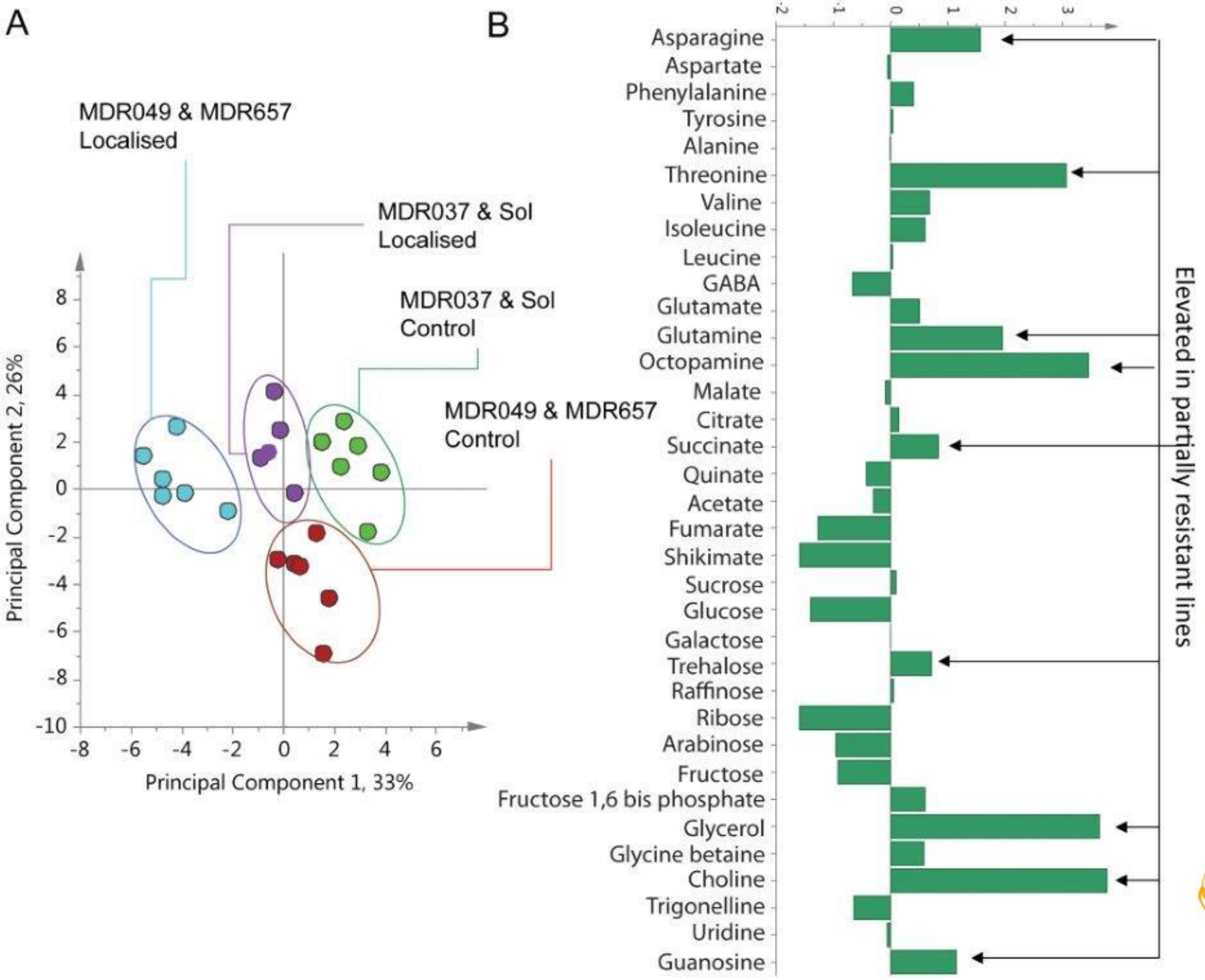


Reduced fecundity of *R. padi*

no nymphs produced on MDR045



Metabolomic Analysis showed differential changes in plant chemistry with and without aphid damage



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Screening germplasm for resilience to aphids (WP2.3)

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

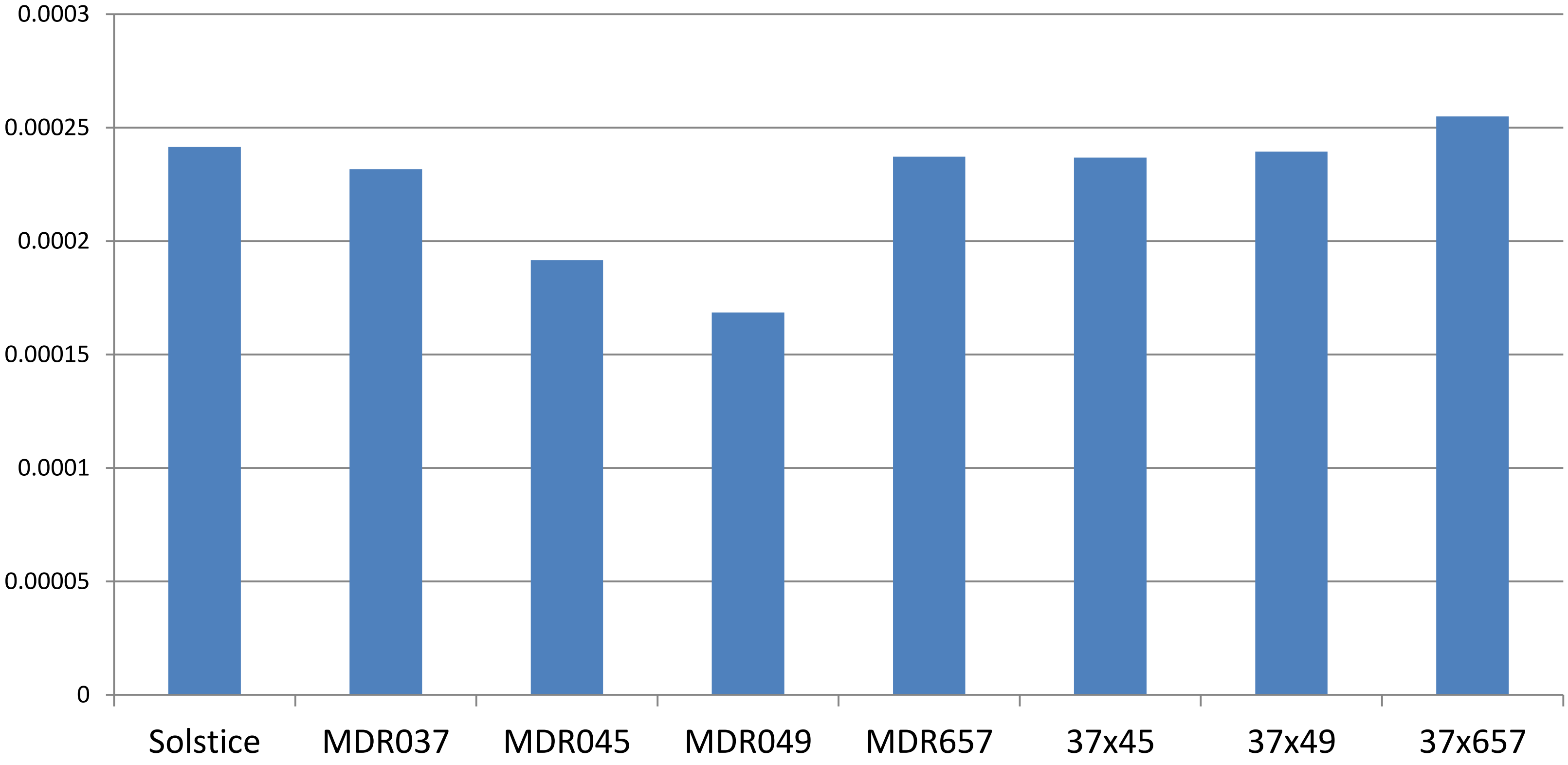
- Crosses made by Mike Hammond-Kosack: MDR037 x MDR045, MDR049 and MDR657

- F1 generations of these crosses have now been tested in the phenotyping screen along with parental lines against both aphid species

- F1 generations of crosses, MDR037 x MDR045, MDR049 and MDR657, tested in the phenotyping screen against both aphid species

Replicate 1					Replicate 2					
WV1				WV17	WV1				WV17	Row 1
WV2				WV18	WV2				WV18	Row 2
		...		C1			...		C1	Row 3
				C2					C2	Row 4
Tray 1	Tray 2	Tray 3	Tray 4	Tray 5	Tray 1	Tray 2	Tray 3	Tray 4	Tray 5	

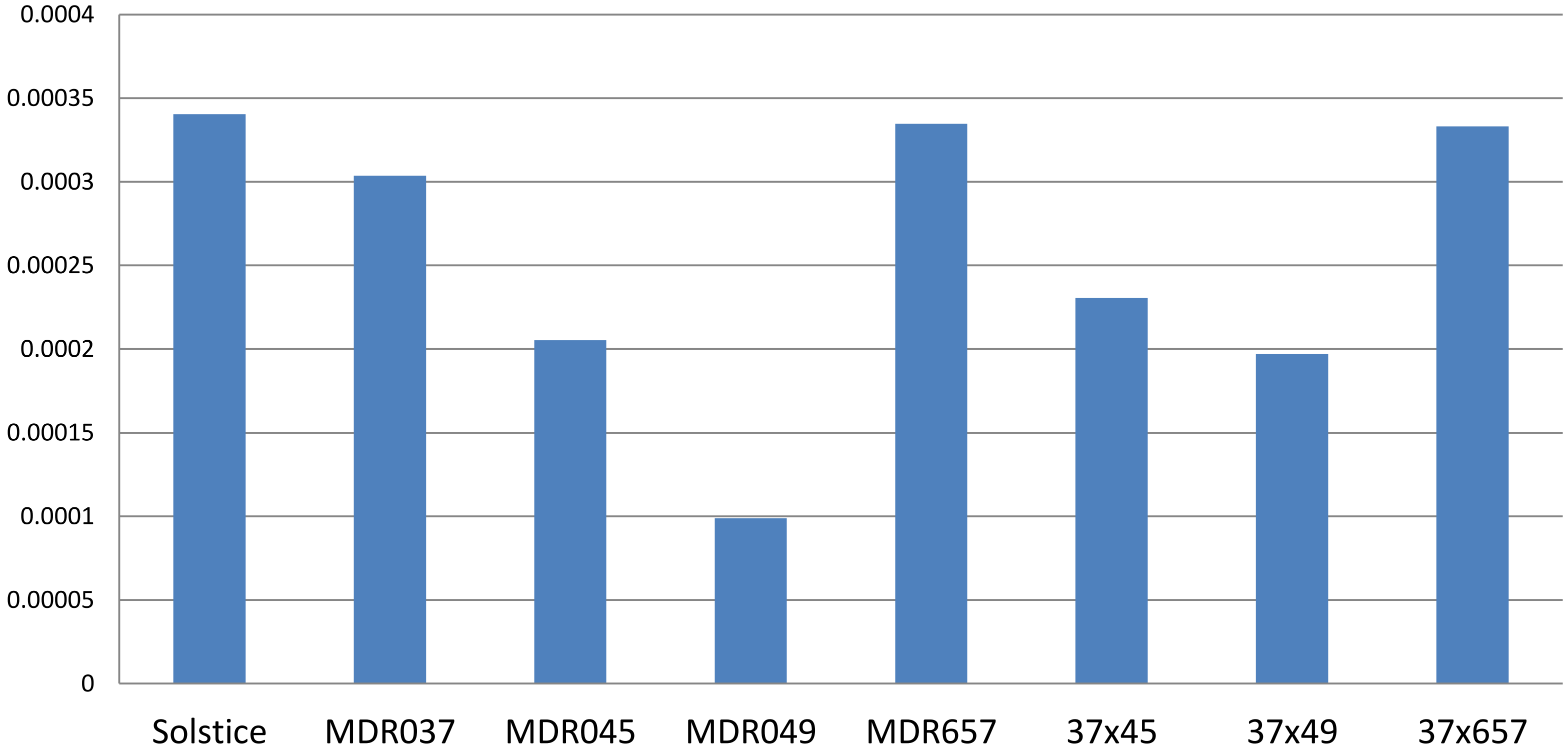
Rhopalosiphum padi mean nymph weight (mg) after 6 days on *T. monococcum* parental lines and F_1 crosses



Plants =10, 10, 10, 10, 10, 9, 8, 10
Aphid batches =10, 7, 10, 10, 10, 7, 8, 10

Nymphs produced on MDR045 and MDR657 – plants older?

Sitobion avenae mean nymph weight (mg) after 7 days on *T. monococcum* parental lines and F_1 crosses



Plants =10, 10, 10, 10, 10, 8, 8, 10
Aphid batches =9, 2, 10, 8, 9, 7, 7, 10

Summary

- Differences observed between responses of aphid species to F1 generations of *T. monococcum* crosses in phenotyping screen, but data limited. Aphid response on some parental lines differed from original findings. MDR049 consistent.

- F2 generations and backcrosses to MDR037 have been generated, or are in progress, and will be screened against both aphid species in the near future

Acknowledgements

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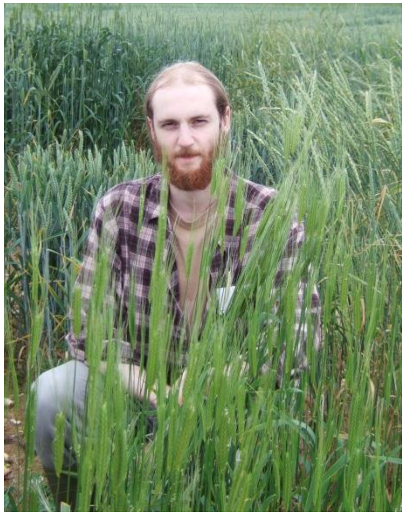
Gia Aradottir
and Mike Hammond-Kosack

Colleagues now moved to other projects



Alex Greenslade

Janet Martin

WISP

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Rothamsted Research
where knowledge grows

WGIN 3
Resistance to take-all and foliar diseases

Vanessa McMillan
Kim Hammond-Kosack

Kostya Kanyuka

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WGIN MM October 2015

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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**

Glasshouse seed multiplication of two *T. monococcum* mapping populations carried out in 2015:

MDR031 (R) x MDR043 (S)
MDR043 (S) x MDR046 (R)

Now at F5, taking forward to F6 in 2016 with enough seed to be multiplied for future field experiments

Resistance to take-all and foliar diseases

Objectives:

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

Pursuing three strategies

620 F_1 seed available

No 1 Take the F_1 Paragon (*ph-1*) x *Tm* MDR grain, excised embryos, grow to young plant stage, wash out root systems, cut roots and treat roots with **colchicine for 7hr,** repot seedlings. We should be able to **identify the plants with successfully doubled chromosomes** because of their **faster rates of growth and development**.

Resistance to take-all and foliar diseases

Objectives:

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

Pursuing three strategies

No 2 **Take *Tm* MDR grain of the key lines of interest**, grow to young plant stage, wash out root systems, cut roots and treat roots with **colchicine for 7hr**, repot seedlings. Then **cross the amphidiploid *Tm* plants** to the Paragon (*ph-1*) mutant.

Resistance to take-all and foliar diseases

Objectives:

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

Pursuing three strategies

No 3 Take *Tm* MDR grain, grow to young plant stage, wash out root systems, cut roots and treat roots with **colchicine for 7hr,** repot seedlings. **Then cross the amphidiploid *Tm* plants to a durum wheat.** We will also try direct *Tm* to durum crosses.

Then cross F_1 *Tm* x *T. durum* plants to Paragon (+/- the *ph-1* locus).

Q – Which durum wheat ?

Resistance to take-all and foliar diseases

Objectives:

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

- Non-host/highly resistant across whole collection in 2015 inoculated field trial - **outcome - no yellow rust despite very high disease pressure (n ~ 200 accessions)**
- Whole collection still to be genotyped to provide fully characterised genetic resource for future studies
- **Will now focus on more promising Watkins lines for analysing yellow rust 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

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			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

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Watkins foliar disease field trial 2015

26th June 2015 1st wheat Long Hoos 4

Yellow rust dominant disease that developed across 2015 field trials

5/10 Watkins lines show some resistance to yellow rust

Watkins line	Field response
203	MR
231	M/MR
610	M/MR
733	0
786	MS (May), MR (June)

Summer 2015

cv. Fielder
Flag leaf = 100% S

Watkins 733
No disease symptoms

Watkins field crossing with cv. Fielder

Watkins line	Yellow rust resistance	Ears crossed	F_1 Grains
18*	MS	7	70
203 (India)	MR	8	31
231 (Hungary)	M/MR	8	54
495*	MS	6	13
610 (Yugoslavia)	M/MR	6	35
733 (Iran)	0	6	46
Totals		**41**	**249**

* Included in crossing as low disease severity in May

Crossing carried out by Mike Hammond-Kosack

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	
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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

Possible opportunity :Watkins 231 x Paragon population could be field sown in spring 2016 and then phenotyped ←

Watkins foliar disease trial summary

- 5/10 lines very susceptible to yellow rust – escaped disease in 2008 or different YR races?

- 5/10 lines show some resistance:

  1/5 = no disease response, 4/5 = M or MR

- Field crossing carried out between Watkins and cv. Fielder

- Plant samples taken on 13th July to be assessed for take-all

– evidence for an induced resistance response or not?

Next steps – Watkins foliar disease

- Plant samples to be assessed for take-all disease over winter months and data to be fully statistically analysed

- Watkins lines were hand harvested in August 2015 and repeat trial drilled for 2015/2016 field season

- Foliar diseases will be scored in spring and summer 2016

- Mapping populations to be sown in glasshouse and progressed to F_2

  203 (India) x Fielder
  610 (Yugoslavia) x Fielder
  733 (Iran) x Fielder

- 231 (Hungary) x Paragon field sow the available mapping population if at an appropriate generation

Many thanks to

ROTHAMSTED
RESEARCH

Kim Hammond-Kosack
Gail Canning

<u>PhD students</u>
Sarah-Jane Osborne
Joseph Moughan

<u>Undergraduate summer students</u>
Erin Baggs
Eleanor Leane
Tessa Reid

Mike Hammond-Kosack – crossing and introgression
Lucy Nevard – seed preparation

Rodger White - statistics

RRes farm and glasshouse staff

Sarah Holdgate (NIAB)

Simon Orford (JIC)

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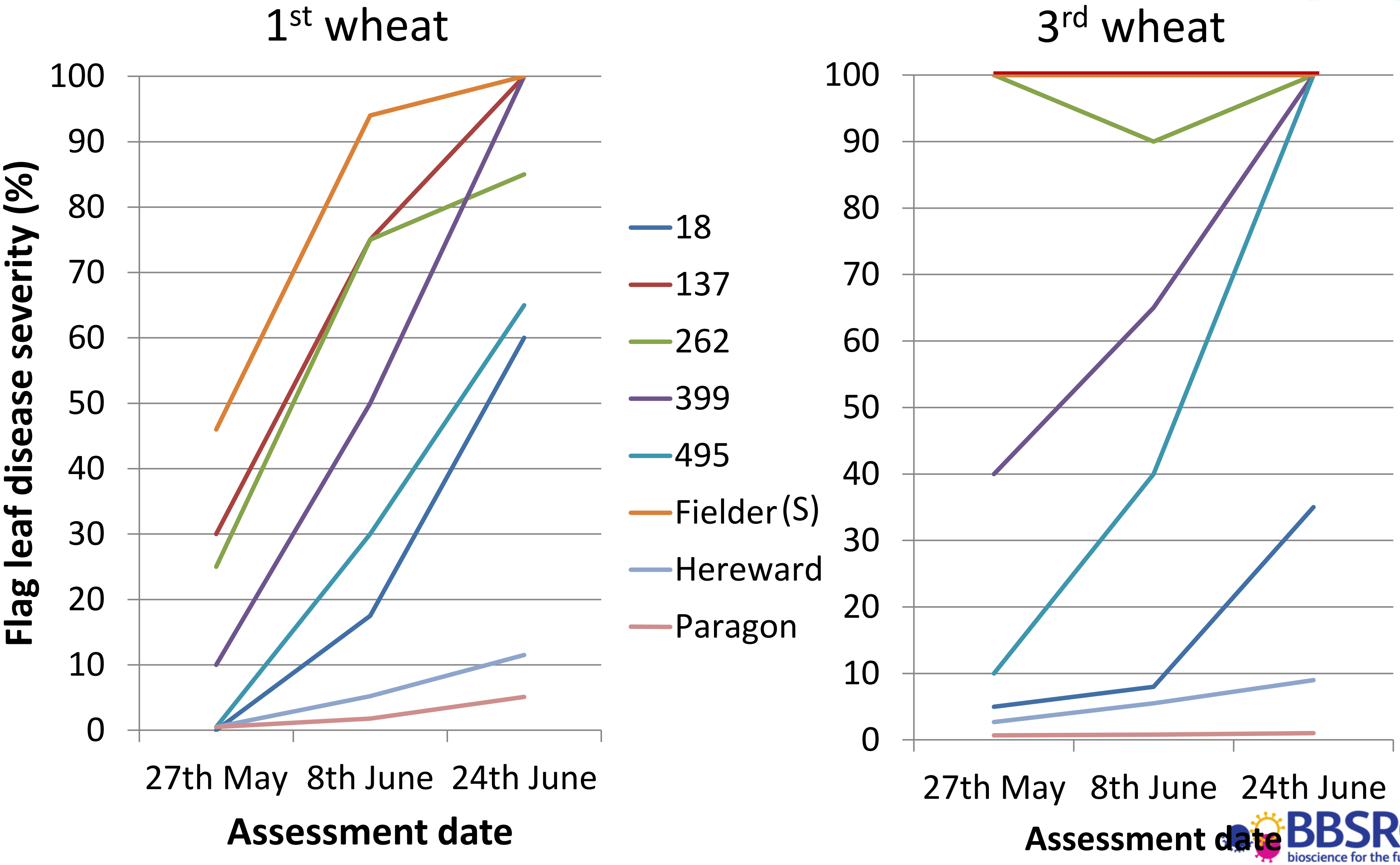
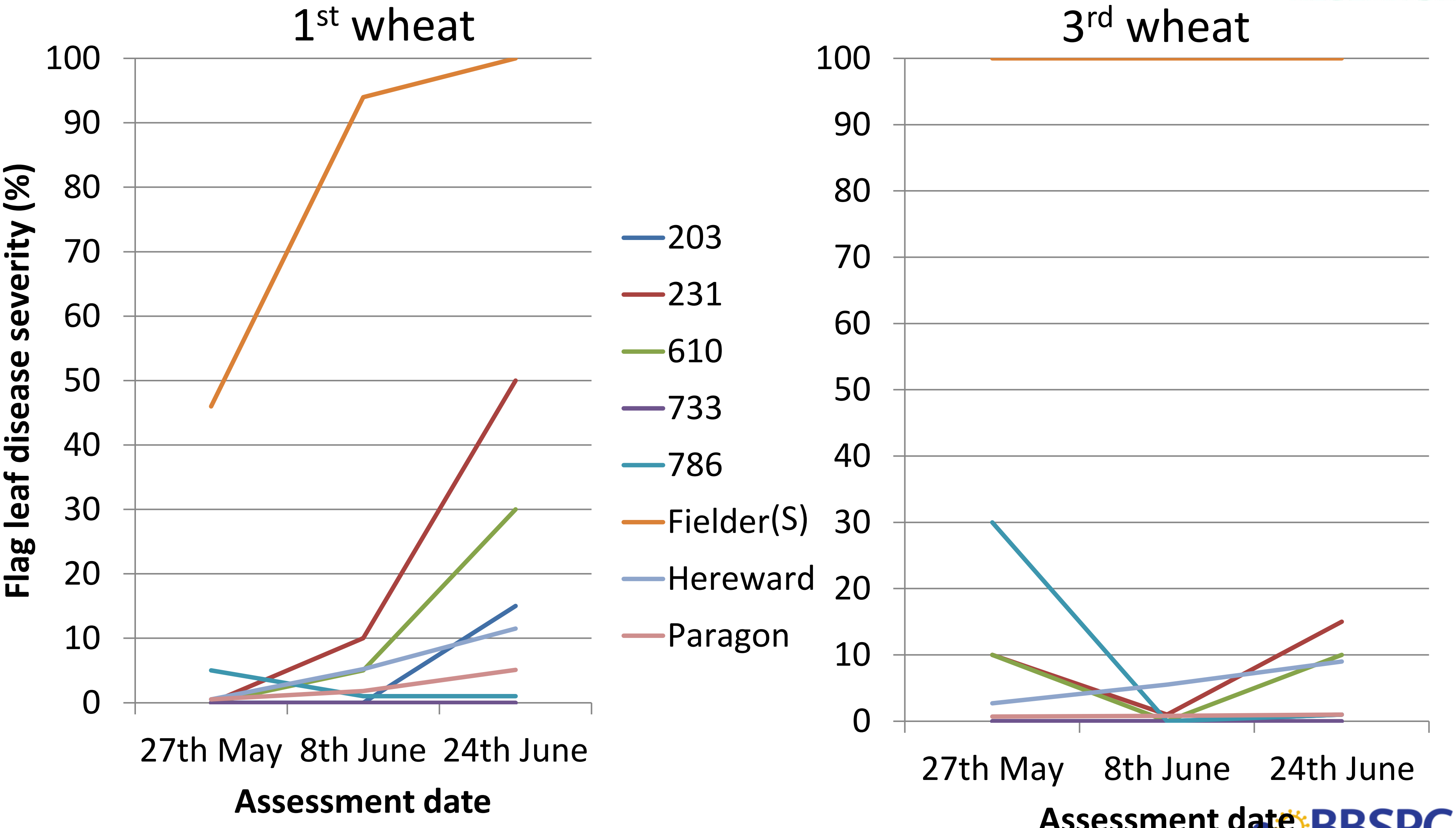
5/10 Watkins lines very susceptible to yellow rust



5/10 Watkins lines show some resistance to yellow rust



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Exome Capture

Kim Hammond-Kosack

MYcroarray

Ann Harbor, Michigan, USA

28th October 2015

Exome Capture

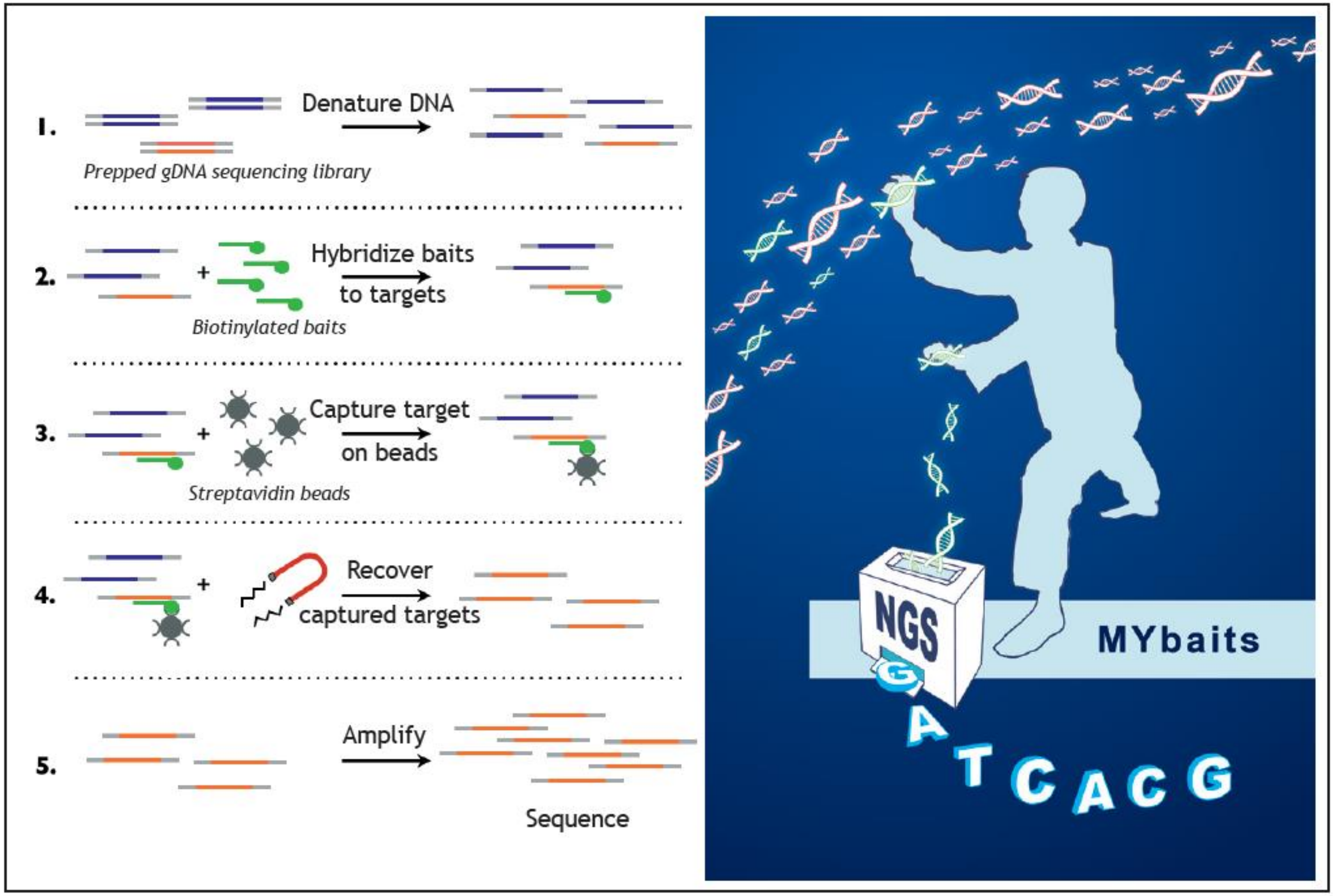
The overall goal is to use exome capture to identify genetic variation in candidate or known genes that are responsible for the desired trait (s)

Exome capture (WP 4.2, 4.4 and Milestones 18)
A designated group of WGIN scientists will interact with the company MYcroarray to decide on the best way to represent wheat genes on the **20,000 bit array.**

Focus : promoter analysis

Custom bait libraries for target sequencing

Mybaits is a fully customisable liquid-phase DNA capture system
for targeted sequencing



High percentage of reads on target.

Summary of exome capture discussions @ WGIN Stakeholder meeting 16th April 2015

Focus : promoter sequences (~1kb) – NOVELTY

A, B and D genome sequences to be individually captured

- Bioinformatics will be quite challenging to ID the 3 homoeologous promoters

Summary of exome capture discussions @ WGIN Stakeholder meeting 16th April 2015

Design: 120-mers across each promoter,
each overlapping by 60 bp (i.e. 2-fold coverage)
16 probes per promoter (960 bp)
48 probes to cover A, B and D promoters / gene
416 x 3 promoters
Or some only evaluated for 1 homoeologue

Need to include published positive controls
to validate the technology
For example - *ppd1, vrn1A*

Need to remove
MITEs - miniature inverted-*repeat* transposable elements
from the probe sets developed

Summary of exome capture discussions @ WGIN Stakeholder meeting 16th April 2015

Developing the list of 96 cultivars

Need to relate to ongoing / previous wheat projects (WGIN and beyond)

Generic Resources

Avalon
Cadenza
Paragon
Chinese Spring
Kronos (tetraploid)
Diploids

Alchemy, Hereward, **Rialto**, Robigus, Savannah and Xi19
- Wingfield et al (2012) PBJ study

Specific traits

Garcia
Watkins 777

Summary of exome capture discussions @ WGIN Stakeholder meeting 16th April 2015

Developing the promoter – gene list – 416 x 3 genomes

Traits

1. **Yield resilience**
2. **Grain quality**
3. **Biotic stress – fungi and insects**
4. **Abiotic stress – drought, high temp**
5. **Nutrient use efficiency**
6. **Canopy development**
7. **Flower biology**
8. **Root architecture**

50 nominated promoters per trait category

Who to be involved ?

Yield resilience	Cristobal Uauy*, need a 2nd
Grain quality	Peter Shewry, Kay Trafford (NIAB), Rowan Mitchell (RRes)
Biotic stress – fungi and insects	Kim H-K (RRes), Brandt Wulff (JIC), Matthew Moscow (SL), insects ??
Abiotic stress – drought, high temp	John Foulkes, need a 2nd
Nutrient use efficiency	Malcolm Hawkesford and Nottingham
Canopy development / whole plant architecture)	Simon Griffiths, Allison Bentley (NIAB), Andy Phillips*
Flower biology	Zoe Wilson, need a 2nd
Root architecture	Malcolm Bennett, need a 2nd

Breeding community - nominations?

* BBSRC BBR wheat tilling project and exome capture

Taken a short pause - Why ?

- **Considerably more Chinese Spring sequencing data to be released into the public domain in Nov 2015**
- **To be uploaded into ENSEMBL (EBI)**
- **Transfer annotations from any public source onto this new genome release once a quarter**
- **EBI contacts are Paul Kersey and Dan Bolser**

WGIN Promoter capture experiment

Focus : promoter sequences (~1kb) – NOVELTY

A, B and D genome sequences to be individually captured

- Bioinformatics will be quite challenging to ID the 3 homoeologous promoters

Advantages going forward will be

- **Nominators just need to identify the correct locus ID in ENSEMBL wheat and enter this into the Excel sheet and also view the promoter sequence.**

- **Homoeologous promoter identification should be easier, because of the wheat chromosome maps in ENSEMBL**

- **The RREs bioinformatician can directly use the ENSEMBL software to pull back the correct 1kb promoter sequences using the locus ID number.**

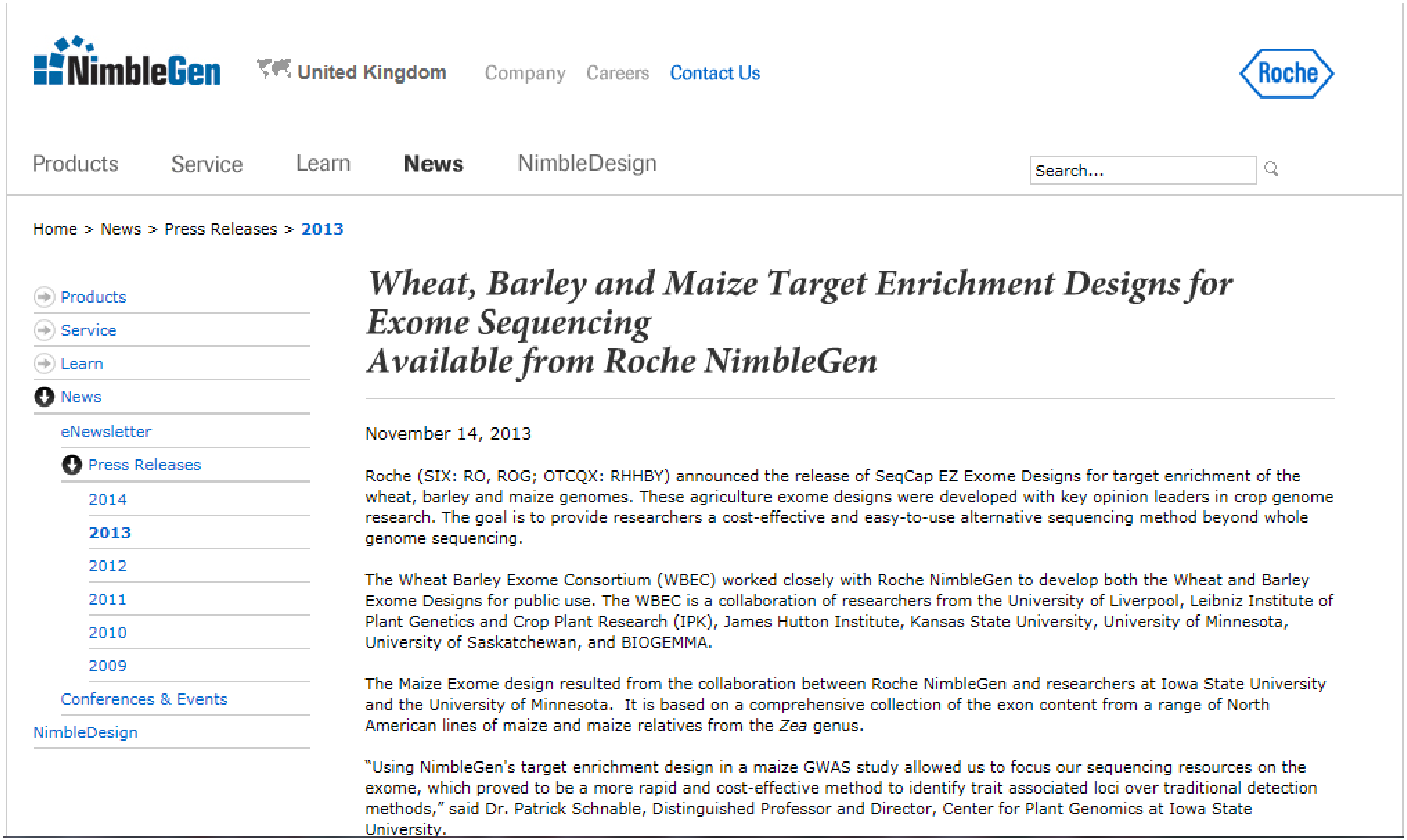
Exome capture – next steps

4-6 individuals interested in taking this WP forward

- finalise the oligo design method
- select the wheat gene list
- select the 96 wheat genotypes

Series of Skype calls / WORKSHOP

Interact with the BBSRC funded BBR project which include some exome capture for wheat (Uauy and Philips)



Wheat, Barley and Maize Target Enrichment Designs for Exome Sequencing Available from Roche NimbleGen

November 14, 2013

Roche (SIX: RO, ROG; OTCQX: RHHBY) announced the release of SeqCap EZ Exome Designs for target enrichment of the wheat, barley and maize genomes. These agriculture exome designs were developed with key opinion leaders in crop genome research. The goal is to provide researchers a cost-effective and easy-to-use alternative sequencing method beyond whole genome sequencing.

The Wheat Barley Exome Consortium (WBEC) worked closely with Roche NimbleGen to develop both the Wheat and Barley Exome Designs for public use. The WBEC is a collaboration of researchers from the University of Liverpool, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), James Hutton Institute, Kansas State University, University of Minnesota, University of Saskatchewan, and BIOGEMMA.

The Maize Exome design resulted from the collaboration between Roche NimbleGen and researchers at Iowa State University and the University of Minnesota. It is based on a comprehensive collection of the exon content from a range of North American lines of maize and maize relatives from the *Zea* genus.

"Using NimbleGen's target enrichment design in a maize GWAS study allowed us to focus our sequencing resources on the exome, which proved to be a more rapid and cost-effective method to identify trait associated loci over traditional detection methods," said Dr. Patrick Schnable, Distinguished Professor and Director, Center for Plant Genomics at Iowa State University.

WGIN3 project

The overall goal is to exome capture to identify genetic variation in candidate or known genes that are responsible for the desired trait (s)

Exome capture (WP 4.2, 4.4 and Milestones 18)
A designated group of WGIN scientists will interact with the company MYcroarray to decide on the best way to represent wheat genes on the **20,000 bit array.**

This will be done via a series of Skype meetings held during months 1-3.

A workshop will be held **to priorities the gene list and the 96 wheat genotypes** to be tested.

A pilot experiment will be done to ensure the DNA is of the correct quality to ensure success.

The full sample set will be sent for the capture using the most appropriate secure carrier.

A wheat example from Andy Phillips@RRes

MYcoarray helped design the oligo array for ~1700 wheat genes and made the oligos,

The array “design“ was very simple – 120-mers across the whole of each CDS, each overlapping by 60 bp (ie 2-fold coverage). But this naïve design resulted in some variation in capture efficiency .

Used a single set of oligos for each gene, based on a single homoeologue. The on-target homoeologue represented ~50% of all reads, with the other two homoeologues having ~25% each, on average.

Additional comments

A minimum of 20,000 baits – corresponding to ~1200 coding sequences of average length 1kb.

You will achieve a **more comprehensive capture by using genomic sequence not CDS** for oligo design (we lost small exons in our captures) so that you can add some flanking intron sequence **(and promoter, probably important for surveying natural variation).**