WGIN 3 Management meeting

Introduction to the new project

Wheat
Genetic
Improvement
Network

Kim Hammond-Kosack

Rothamsted Research

defra
Department for Environment
Food and Rural Affairs

17th July 2015
RRes

WGIN phase 3 (WGIN3)

March 2015 - Feb 2017

Project title

Defra Wheat Genetic Improvement Network

- Improving the resilience of the wheat crop through genetics and targeted traits analysis

Wheat Genetic Improvement Network (WGIN3) 2015-2017

Red text -
new to WGIN3

WP1 Management meetings – The Network

WP3 Tools and Resources

Maintain and further develop, mapping pop^n, Watkins/Gediflux, *T. monococum* collections (3.1)

Create an A x C NIL TILLING pop^n (3.2)

T. monococcum introgression (3.3)

WPs 2, 3 & 4 Targeted Traits

Aphid resistance (2.2)

Take-all resistance (2.2, 3.4)

Septoria and yellow rust resistance (2.2)

Yield and quality resilience (2.2, 3.4)

Yield components (2.2)

Drought tolerance (2.2, 3.4)

Root system function (3.4)

WPs 2 & 4 Genetic and QTL analyses

For each of the targeted traits
Gene-specific marker development (2.4)

Sub-contractors – WP1.3 & 4.2 NGS genome / exome analyses

WP1 Enhancing the network and communication of results

Website (1.2)
Annual Stakeholders forum (1.1)
International collaborations (1.4)
Publications + data deposits (1.4)

Electronic Newsletter (1.4)
Focussed workshops (1.1)
Public outreach
Industry-led forum (1.5)

WGIN 3 project partners

John Innes Centre – Simon Griffiths

Rothamsted Research - Kim Hammond-Kosack

Two sub- contractors

Bristol Genomics Facility
Univ. Bristol, UK

MYcroarray
Michigan, USA

Genotyping using Affymetrix arrays

Allelic variation via Exome Capture

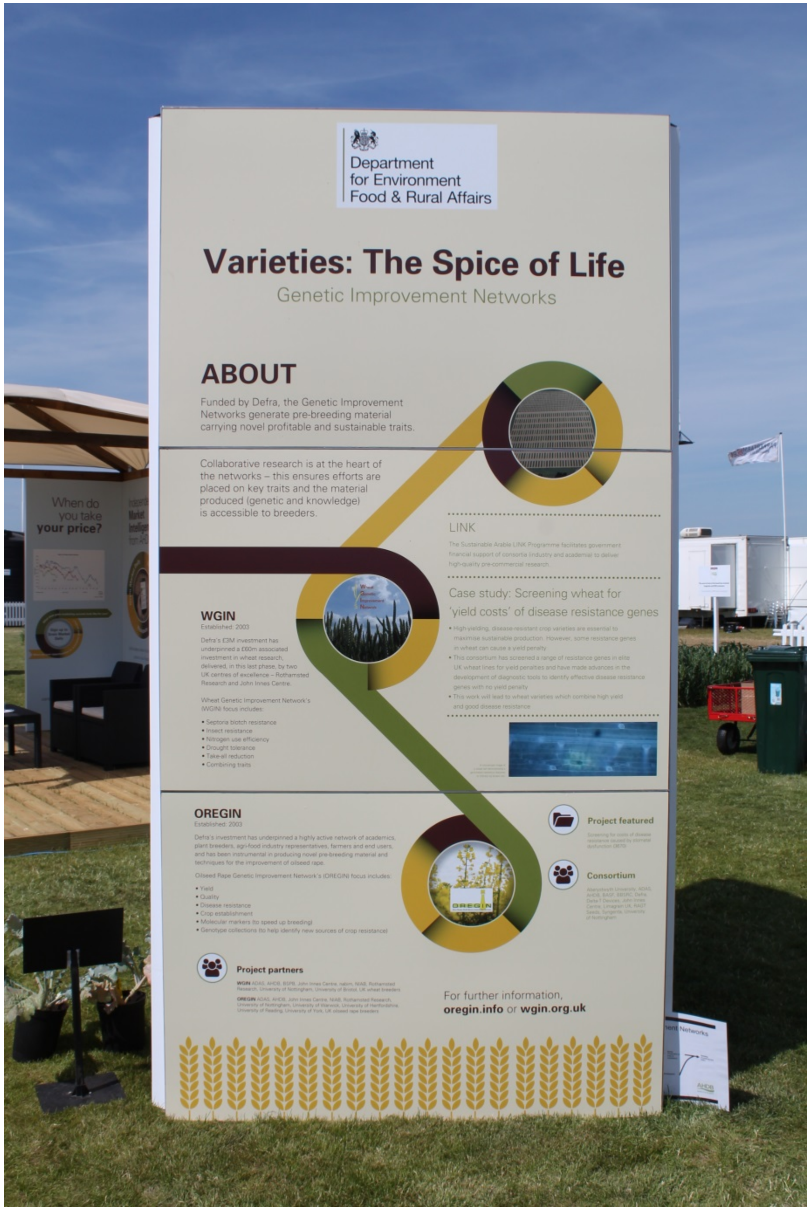
Twenty one project milestones

Done

1	(March 15)	First stakeholder meeting - JIC
2	(February 15)	Development of new near isogenic lines.
3	(throughout project)	Further maintenance and distribution of Avalon x Cadenza doubled haploid population.
4	(Feb 15)	Genetic characterisation of Paragon mutants.
5	(March 15)	Identification of useful genetic variation in Watkins population.
6	(Apr 15)	Development of new mapping populations.
7	(June 15)	First Interim written report to Defra
8	(Sept 15)	Resistance to cereal aphids, information to establish the likely genetic basis of resistance to cereal aphid (Sept 15)
9	(Aug 15)	Development of new QTL for yield at low and high N input
10	(Sept 15)	Information on stability of yield and nitrogen use efficiency parameters for elite varieties
11	(Oct 15)	Collection of data on variation in canopy longevity and nitrogen remobilisation
12	(Dec 15)	Evaluation of lines with good bread-making properties
13	(Dec 15)	Second stakeholder meeting
14	(Jan 16)	Second Interim written report to Defra/Project evaluation
15	(Feb 16)	Improvement of water use efficiency and drought tolerance traits
16	(Feb 16)	Take-all disease, genetic basis, introgression of lines harbouring resistance
17	(March 16)	Introgression of extreme resistance to Septoria tritici from T. monococcum
18	(Apr 16)	Information on germplasm with new important traits.
19	(Summer 16)	Grain Archiving: from each plot of the annual diversity and Avalon x Cadenza field
20 and 21	(Dec 16)	Third Stakeholder meeting and 21. Report (Interim or final)

Plus a detailed Gantt chart covering all activities linked to these milestone

Publicising the WGIN and OREGIN on the AHDB stand at Cereals 2015



Department for Environment Food & Rural Affairs

Varieties: The Spice of Life

Genetic Improvement Networks

ABOUT

Funded by Defra, the Genetic Improvement Networks generate pre-breeding material carrying novel profitable and sustainable traits.

Collaborative research is at the heart of the networks – this ensures efforts are placed on key traits and the material produced (genetic and knowledge) is accessible to breeders.

LINK

The Sustainable Arable LINK Programme facilitates government financial support of consortia (industry and academia) to deliver high-quality pre-commercial research.

Case study: Screening wheat for 'yield costs' of disease resistance genes

- High-yielding, disease-resistant crop varieties are essential to maximise sustainable production. However, some resistance genes in wheat can cause a yield penalty
- This consortium has screened a range of resistance genes in elite UK wheat lines for yield penalties and have made advances in the development of diagnostic tools to identify effective disease resistance genes with no yield penalty
- This work will lead to wheat varieties which combine high yield and good disease resistance

WGIN

Established: 2003

Defra's £3M investment has underpinned a £60m associated investment in wheat research, delivered, in this last phase, by two UK centres of excellence – Rothamsted Research and John Innes Centre.

Wheat Genetic Improvement Network's (WGIN) focus includes:

- Septoria blotch resistance
- Insect resistance
- Nitrogen use efficiency
- Drought tolerance
- Take-all reduction
- Combining traits

OREGIN

Established: 2003

Defra's investment has underpinned a highly active network of academics, plant breeders, agri-food industry representatives, farmers and end users, and has been instrumental in producing novel pre-breeding material and techniques for the improvement of oilseed rape.

Oilseed Rape Genetic Improvement Network's (OREGIN) focus includes:

- Yield
- Quality
- Disease resistance
- Crop establishment
- Molecular markers (to speed up breeding)
- Genotype collections (to help identify new sources of crop resistance)

Project featured

Screening for costs of disease resistance caused by stomatal dysfunction (3670)

Consortium

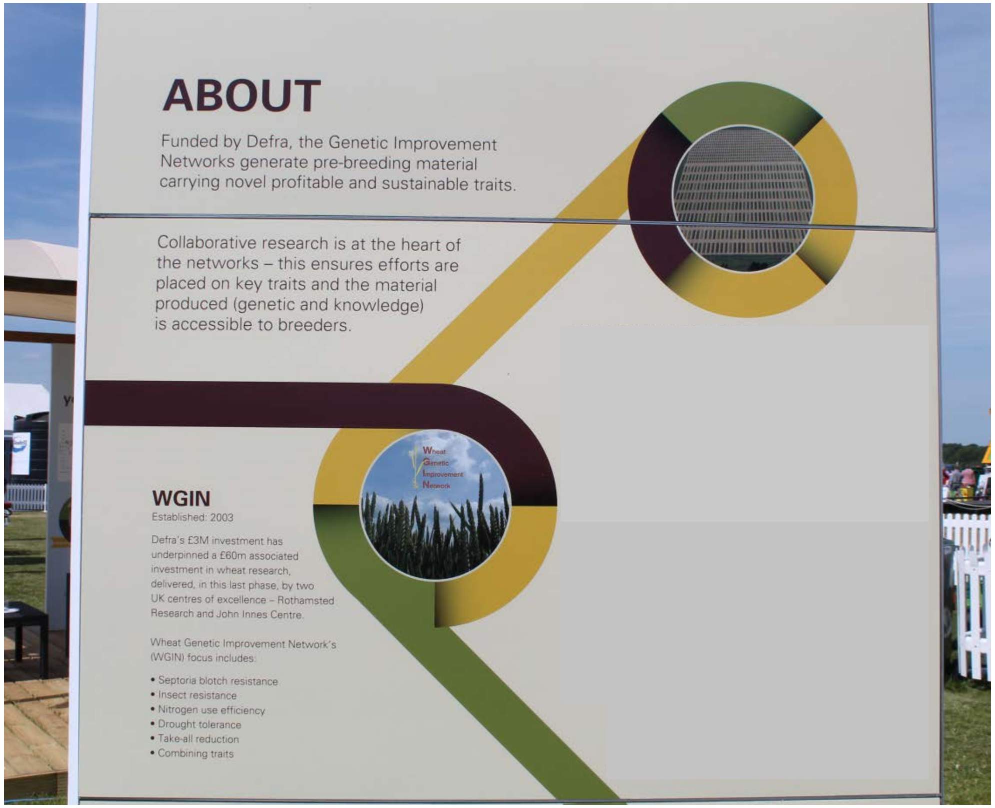
Aberystwyth University, ADAS, AHDB, BASF, BBSRC, Defra, Delta-T Devices, John Innes Centre, Limagrain UK, RAGT Seeds, Syngenta, University of Nottingham

Project partners

WGIN ADAS, AHDB, BSPB, John Innes Centre, nabim, NIAB, Rothamsted Research, University of Nottingham, University of Bristol, UK wheat breeders

OREGIN ADAS, AHDB, John Innes Centre, NIAB, Rothamsted Research, University of Nottingham, University of Warwick, University of Hertfordshire, University of Reading, University of York, UK oilseed rape breeders

For further information,
oregin.info or **wgin.org.uk**



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WGIN Stakeholder Event November 2015 @Rothamsted

Possible dates

10th November –Tue – NO
20th November - Fri – RES
23rd November – Mon - reserve
24th November – Tue – NO
27th November – Fri - reserve

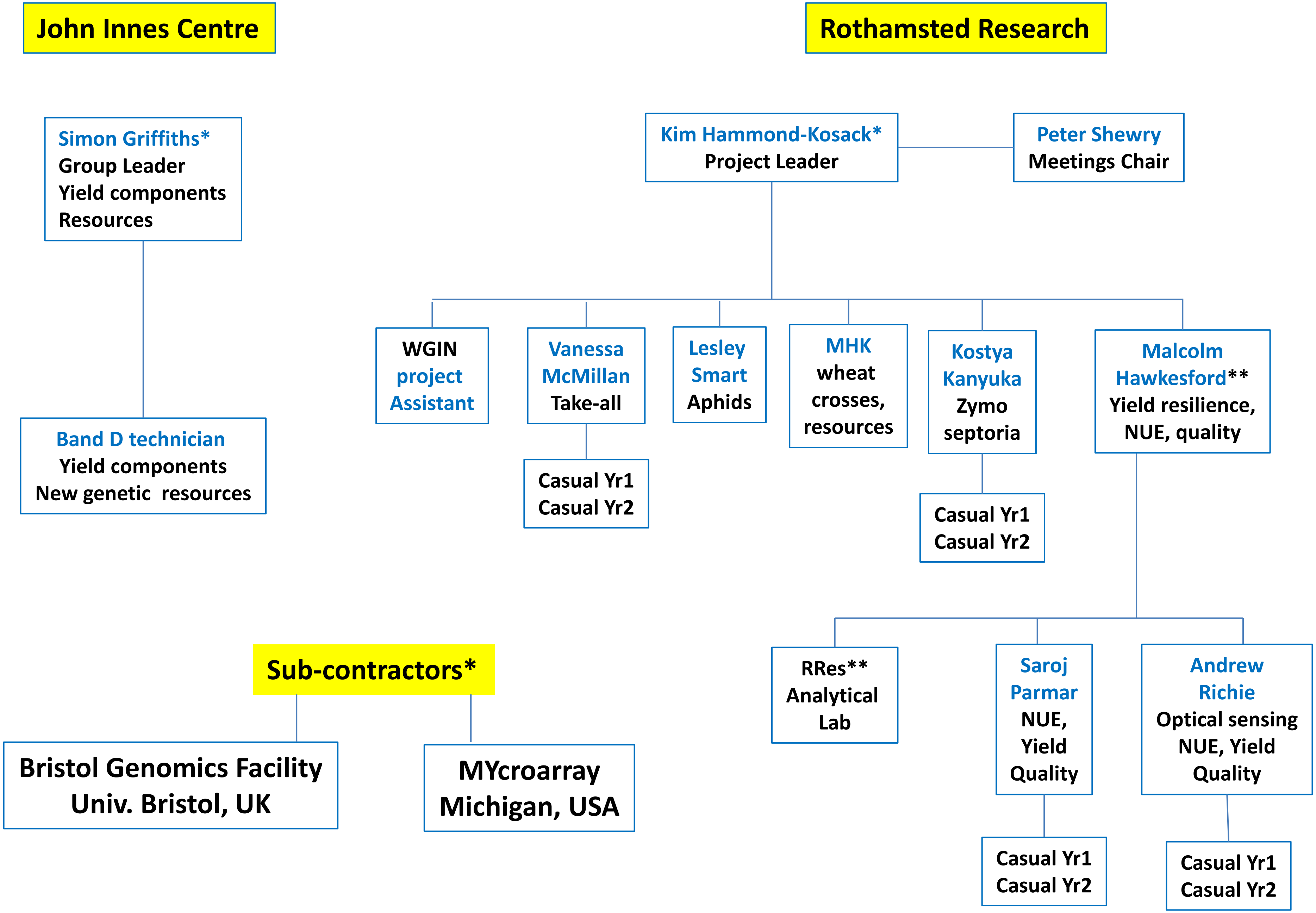
New wheat projects – speakers to invite

Topics for the panel discussion

WGIN phase 3

Improving the resilience of the wheat crop through genetics and targeted traits analysis

THE WGIN3 TEAM



John Innes Centre

Simon Griffiths*
Group Leader
Yield components
Resources

Band D technician
Yield components
New genetic resources

Rothamsted Research

Kim Hammond-Kosack*
Project Leader

Peter Shewry
Meetings Chair

WGIN project Assistant

Vanessa McMillan
Take-all

Casual Yr1
Casual Yr2

Lesley Smart
Aphids

MHK
wheat crosses, resources

Kostya Kanyuka
Zymo septoria

Casual Yr1
Casual Yr2

Malcolm Hawkesford**
Yield resilience, NUE, quality

RRes**
Analytical Lab

Saroj Parmar
NUE, Yield Quality

Casual Yr1
Casual Yr2

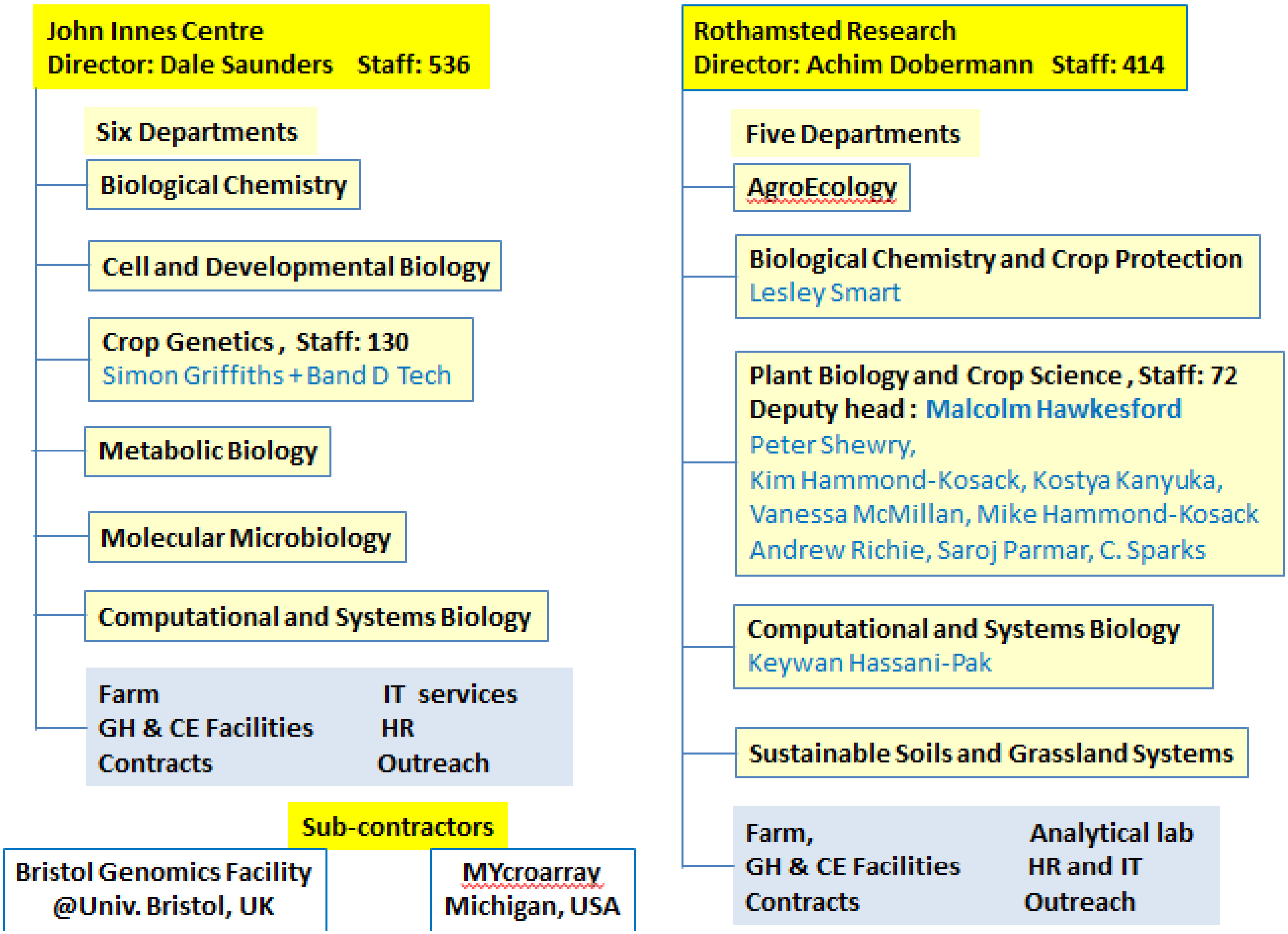
Andrew Richie
Optical sensing
NUE, Yield Quality

Casual Yr1
Casual Yr2

Sub-contractors*

Bristol Genomics Facility
Univ. Bristol, UK

MYcroarray
Michigan, USA



John Innes Centre
Director: Dale Saunders Staff: 536

- Six Departments
  - Biological Chemistry
  - Cell and Developmental Biology
  - Crop Genetics , Staff: 130
Simon Griffiths + Band D Tech
  - Metabolic Biology
  - Molecular Microbiology
  - Computational and Systems Biology
  - Farm, GH & CE Facilities, Contracts, IT services, HR, Outreach

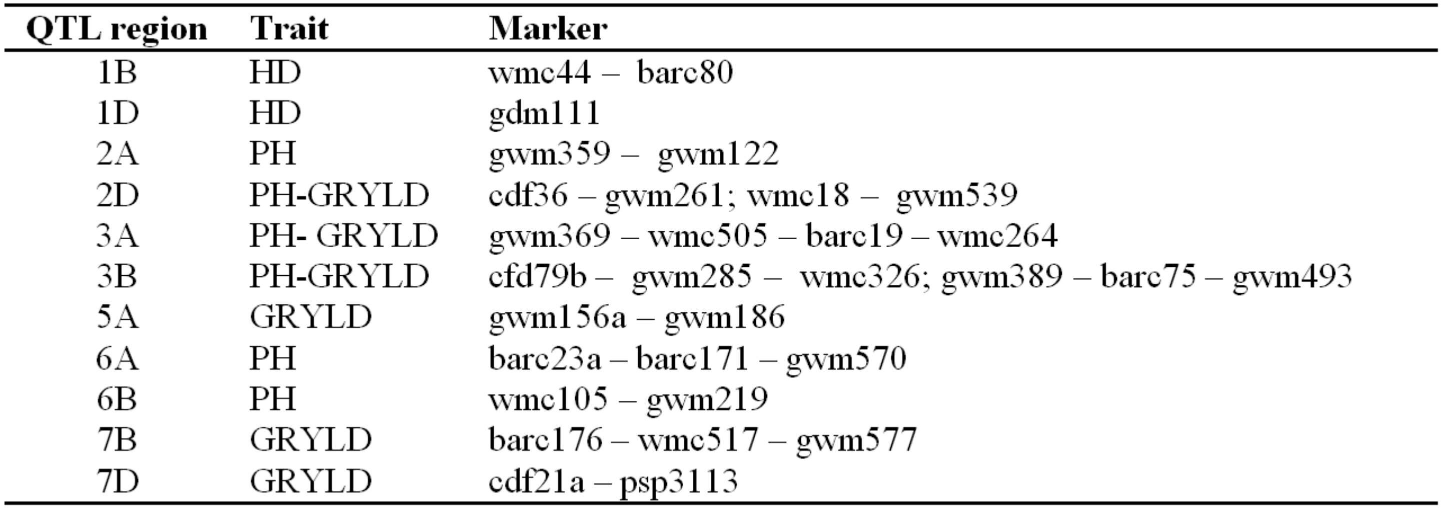
Sub-contractors
- Bristol Genomics Facility @Univ. Bristol, UK
- MYcroarray Michigan, USA

Rothamsted Research
Director: Achim Dobermann Staff: 414

- Five Departments
  - AgroEcology
  - Biological Chemistry and Crop Protection
Lesley Smart
  - Plant Biology and Crop Science , Staff: 72
Deputy head : Malcolm Hawkesford
Peter Shewry,
Kim Hammond-Kosack, Kostya Kanyuka,
Vanessa McMillan, Mike Hammond-Kosack
Andrew Richie, Saroj Parmar, C. Sparks
  - Computational and Systems Biology
Keywan Hassani-Pak
  - Sustainable Soils and Grassland Systems
  - Farm, GH & CE Facilities, Contracts, Analytical lab, HR and IT, Outreach

WGIN NILs analysis

Eleven QTLs, on chromosomes 1B, 1D, 2A, 2D, 3A, 3B, 5A, 6A, 6B, 7B and 7D were chosen as target regions for introgression in our marker assisted backcrossing scheme.



QTL region	Trait	Marker
1B	HD	wmc44 – barc80
1D	HD	gdm111
2A	PH	gwm359 – gwm122
2D	PH-GRYLD	cdf36 – gwm261; wmc18 – gwm539
3A	PH- GRYLD	gwm369 – wmc505 – barc19 – wmc264
3B	PH-GRYLD	cfd79b – gwm285 – wmc326; gwm389 – barc75 – gwm493
5A	GRYLD	gwm156a – gwm186
6A	PH	barc23a – barc171 – gwm570
6B	PH	wmc105 – gwm219
7B	GRYLD	barc176 – wmc517 – gwm577
7D	GRYLD	cdf21a – psp3113

A total of 553 BC_2 NILs were generated (250 and 303 NILs with Avalon and Cadenza background, respectively).

Year: 2013 and 2014
Background: Avalon and Cadenza
Chromosome: 1B, 1D, 2A, 2D, 3A, 3B, 5A, 6A, 6B, 7B and 7D
Allele: Avalon and Cadenza

(5A, 7B and 7D only Avalon background)

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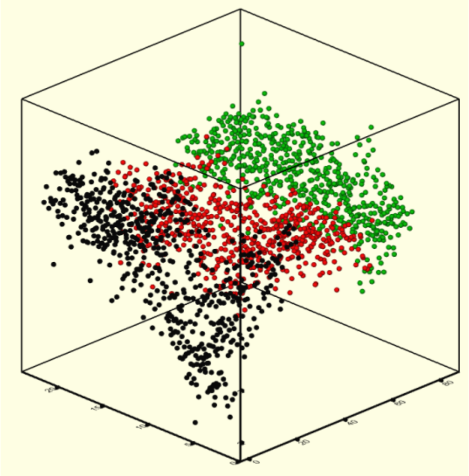
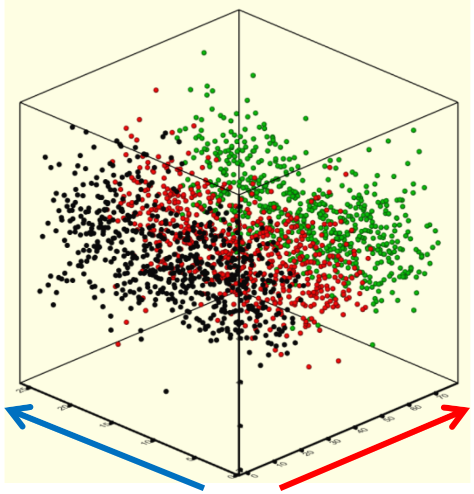
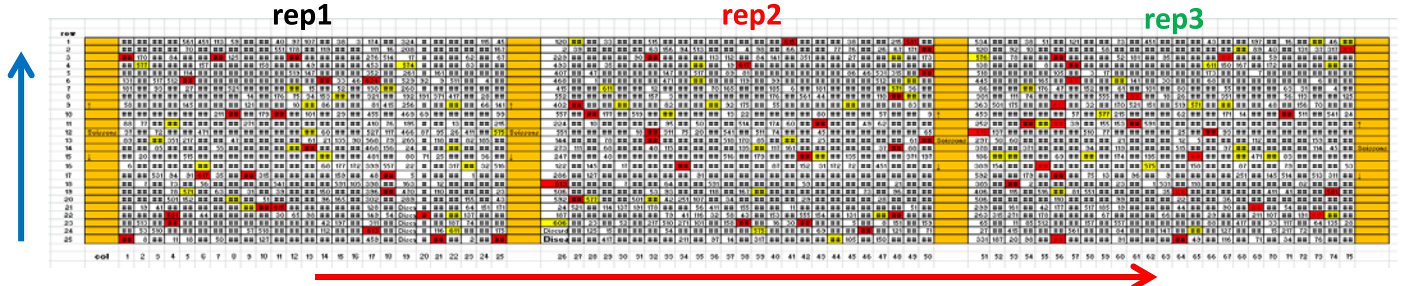
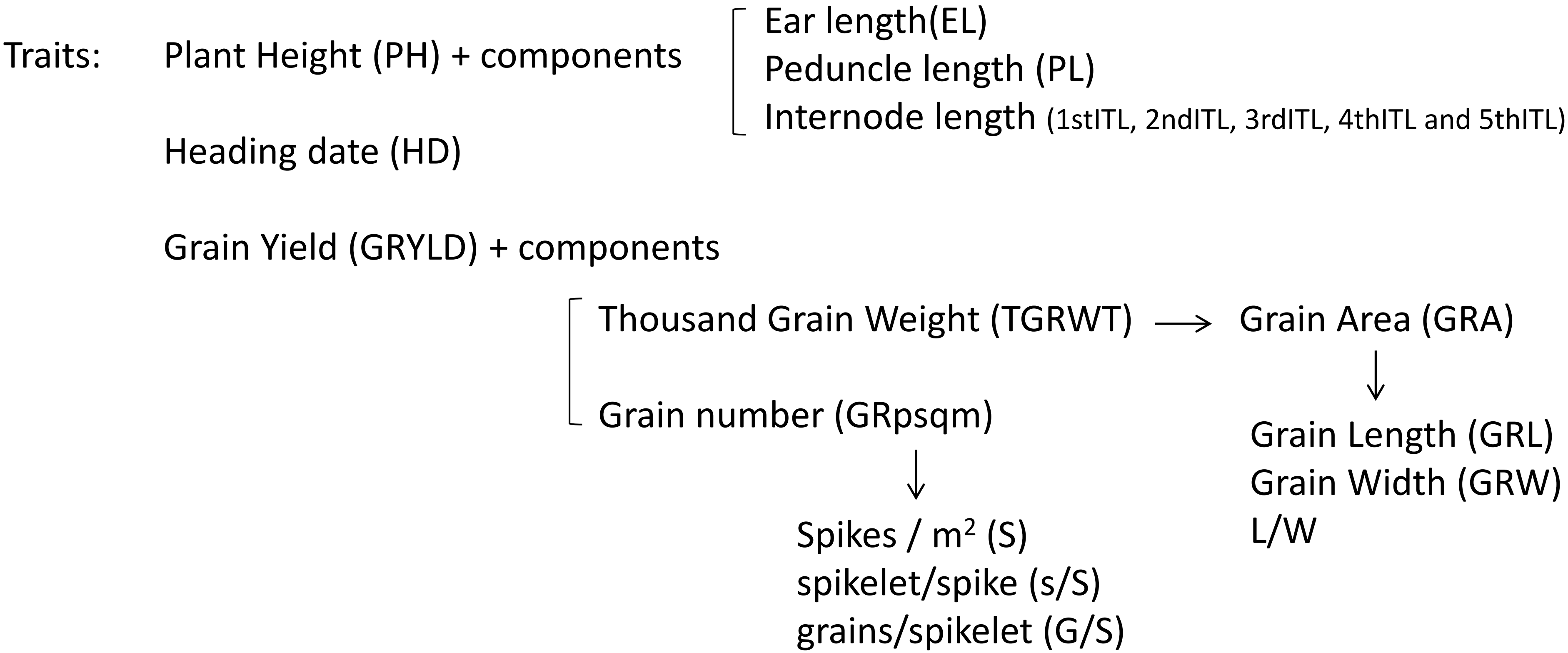
2013



2014







Traits: Plant Height (PH) + components

Ear length(EL)

Peduncle length (PL)

Internode length (1stITL, 2ndITL, 3rdITL, 4thITL and 5thITL)

Heading date (HD)

Grain Yield (GRYLD) + components

Thousand Grain Weight (TGRWT) → Grain Area (GRA)

Grain Length (GRL)

Grain Width (GRW)

L/W

Grain number (GRpsqm)

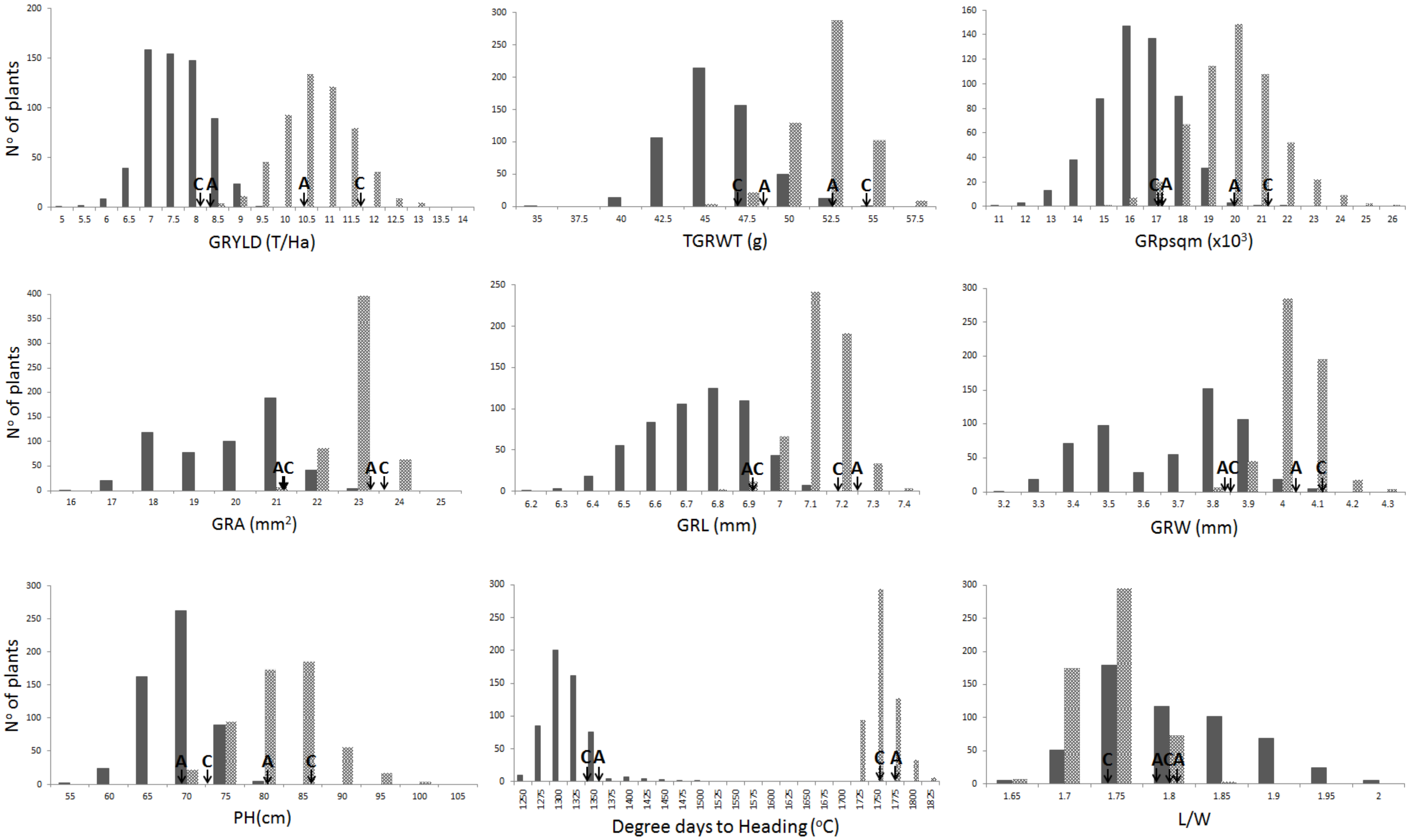
Spikes / m^2 (S)

spikelet/spike (s/S)

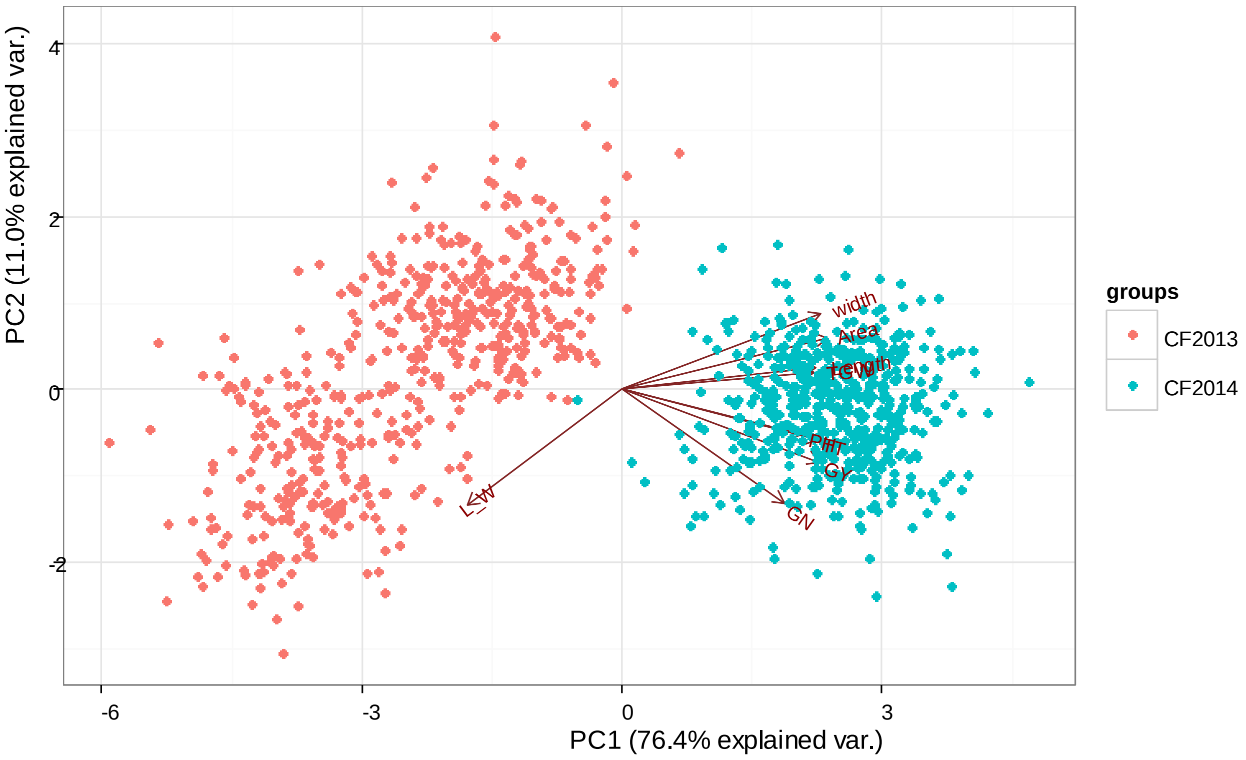
grains/spikelet (G/S)

		Chromosome										
Background	**Allele**	**1B**	**1D**	**2A**	**2D**	**3A**	**3B**	**5A**	**6A**	**6B**	**7B**	**7D**
Avalon	a	3	8	9	37	14	4	8	22	25	4	4
	b	7	5	2	37	12	6	8	8	19	2	6
Cadenza	a	8	19	8	27	29	13	-	26	15	-	-
	b	9	22	5	33	22	17	-	22	27	-	-

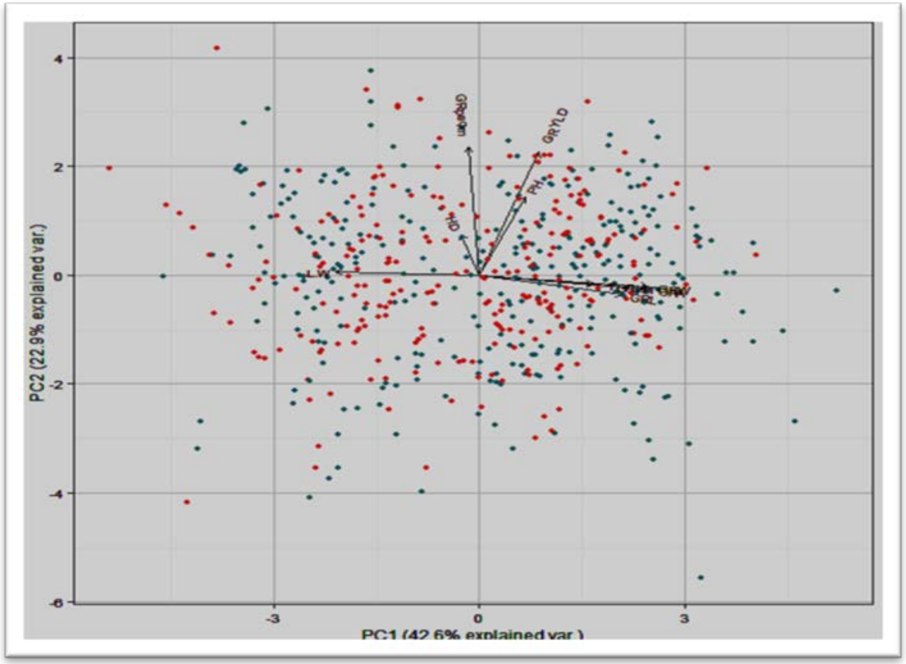
(nº genotype) (5A, 7B and 7D only Avalon background)



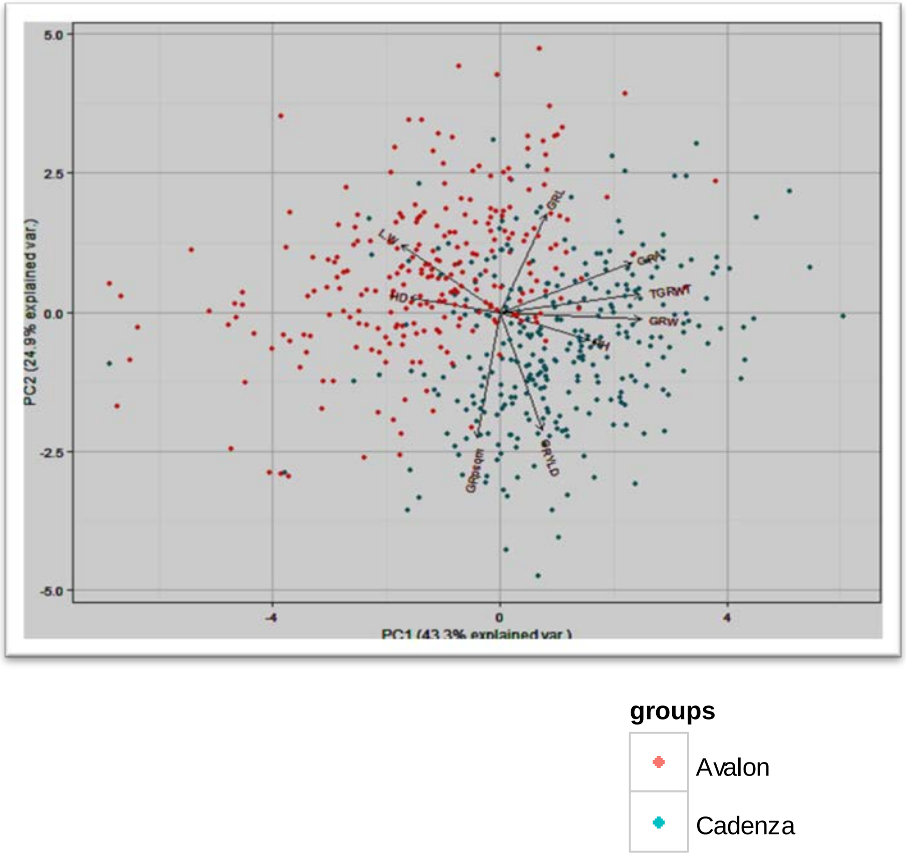
Nº of plants
GRYLD (T/Ha)
C A
A
C
5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10 10.5 11 11.5 12 12.5 13 13.5 14
0 50 100 150 200
TGRWT (g)
C A
A C
35 37.5 40 42.5 45 47.5 50 52.5 55 57.5
0 50 100 150 200 250 300
GRpsqm (x10^3)
CA
A C
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
0 20 40 60 80 100 120 140 160
Nº of plants
GRA (mm^2)
AC
AC
16 17 18 19 20 21 22 23 24 25
0 50 100 150 200 250 300 350 400
GRL (mm)
AC
C A
6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 7 7.1 7.2 7.3 7.4
0 50 100 150 200 250
GRW (mm)
AC
A C
3.2 3.3 3.4 3.5 3.6 3.7 3.8 3.9 4 4.1 4.2 4.3
0 50 100 150 200 250 300
Nº of plants
PH(cm)
A C
A C
55 60 65 70 75 80 85 90 95 100 105
0 50 100 150 200 250 300
Degree days to Heading (ºC)
CA
C A
1250 1275 1300 1325 1350 1375 1400 1425 1450 1475 1500 1525 1550 1575 1600 1625 1650 1675 1700 1725 1750 1775 1800 1825
0 50 100 150 200 250 300
L/W
C
ACA
1.65 1.7 1.75 1.8 1.85 1.9 1.95 2
0 50 100 150 200 250 300

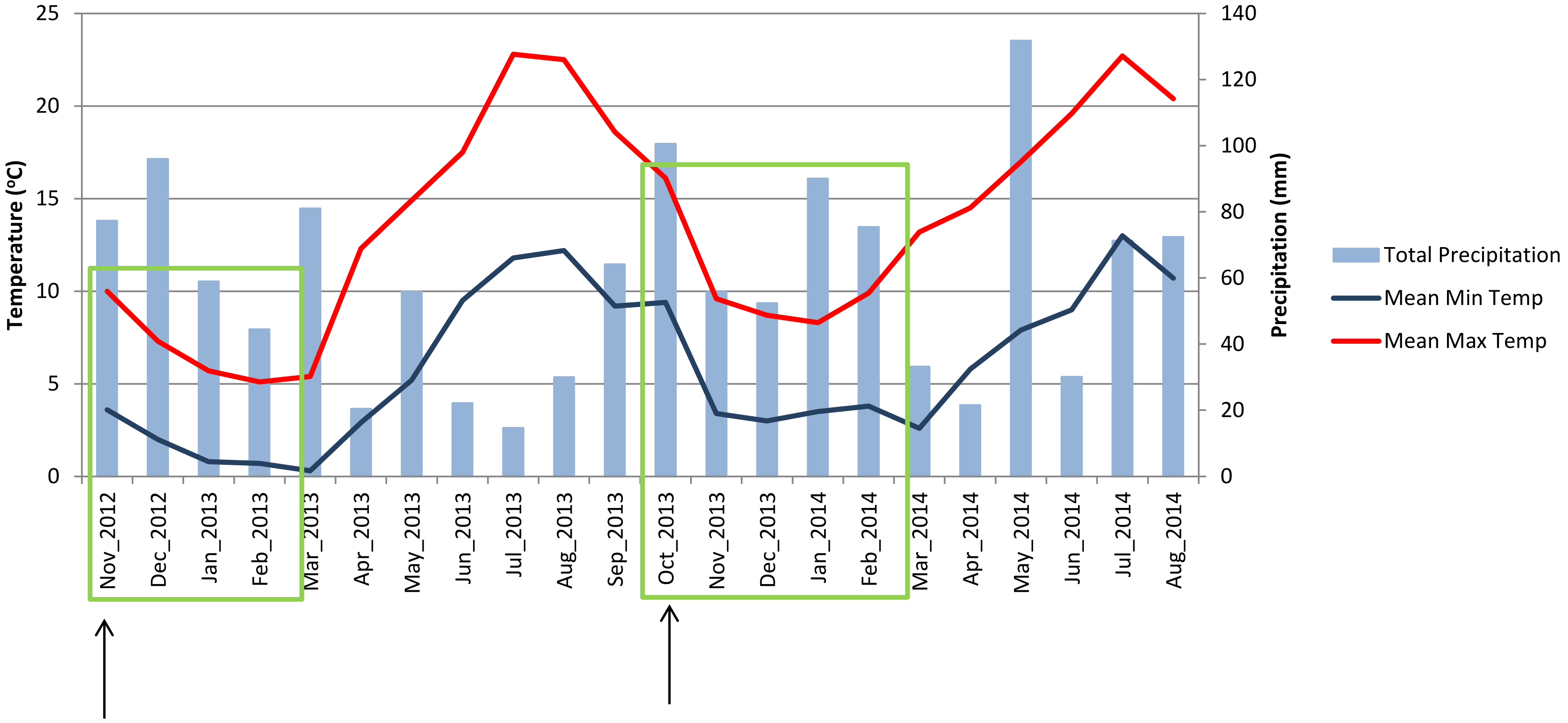

PC2 (11.0% explained var.)
4
2
0
-2
-6
-3
0
3
PC1 (76.4% explained var.)
width
Area
Length
TGW
GY
GN
L_W
groups
CF2013
CF2014

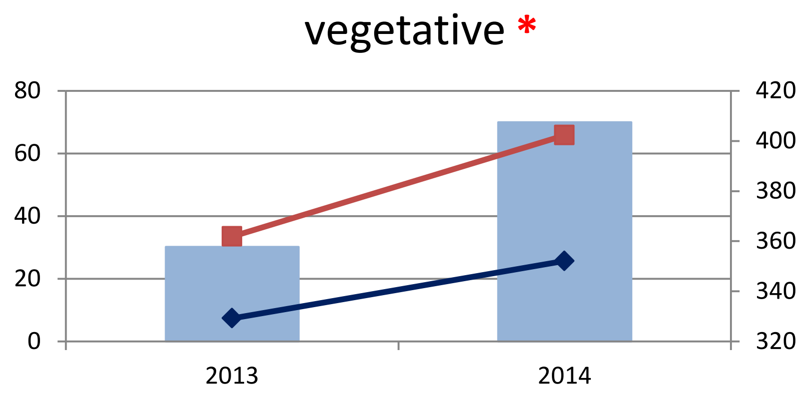
2013

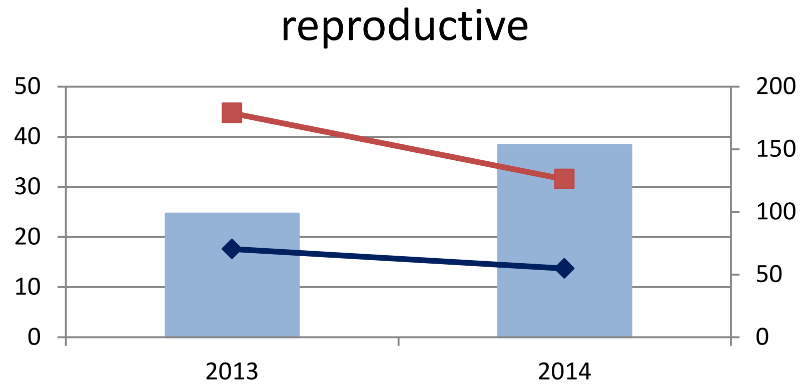

GRpsqm
GRYLD
PH
HD
LW
GRW
GRL
PC2 (22.9% explained var.)
PC1 (42.6% explained var.)
4
2
0
-2
-4
-6
-3
0
3

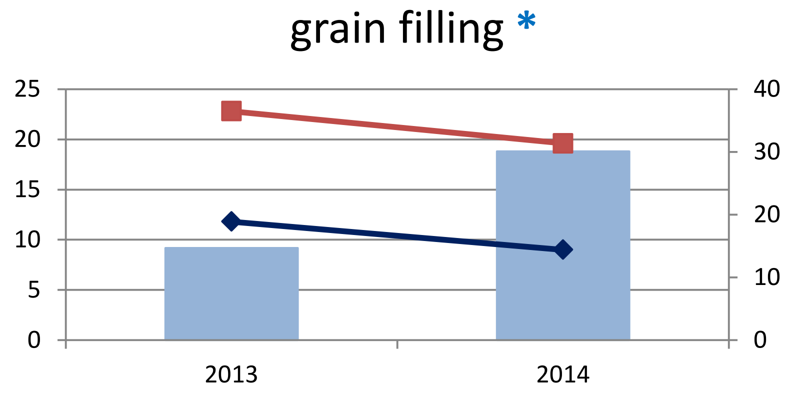
2014


GRL
LW
HD
GRA
TGRWT
GRW
PH
GRpsqm
GRYLD
PC2 (24.9% explained var.)
PC1 (43.3% explained var.)
5.0
2.5
0.0
-2.5
-5.0
-4
0
4
groups
Avalon
Cadenza


Temperature (ºC)
25
20
15
10
5
0
Precipitation (mm)
140
120
100
80
60
40
20
0
Nov_2012
Dec_2012
Jan_2013
Feb_2013
Mar_2013
Apr_2013
May_2013
Jun_2013
Jul_2013
Aug_2013
Sep_2013
Oct_2013
Nov_2013
Dec_2013
Jan_2014
Feb_2014
Mar_2014
Apr_2014
May_2014
Jun_2014
Jul_2014
Aug_2014
Total Precipitation
Mean Min Temp
Mean Max Temp

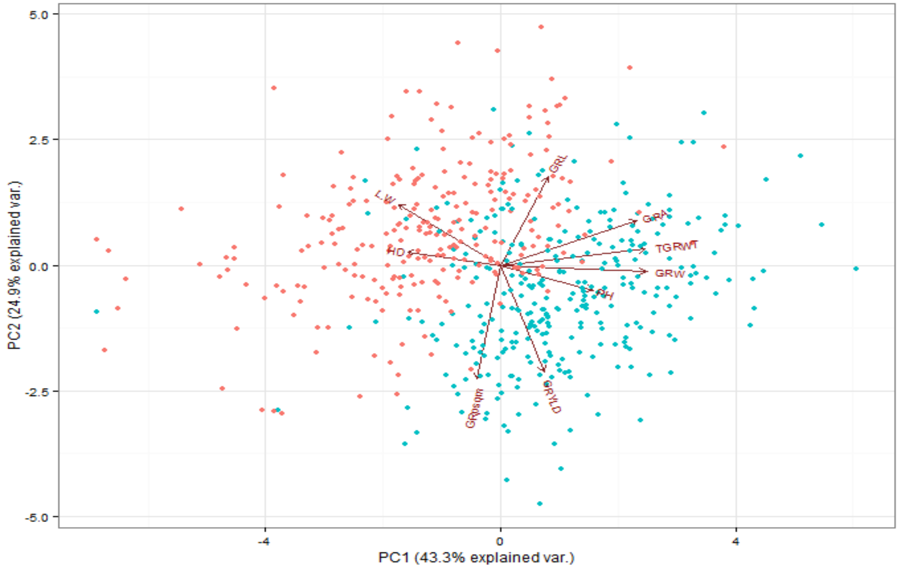

vegetative *


reproductive


grain filling *

Env x background interaction (*)

		Av. backgr.	Cd. Backgr.	Diff.
HD	2013	1368.84	1341.22	27.62
	2014	1782.96	1756.63	26.32
PH *	2013	70.98	72.20	-1.22
	2014	82.16	86.40	-4.24
GY *	2013	7.89	7.95	0.06
	2014	10.51	11.28	0.77
TGW *	2013	46.73	47.09	0.36
	2014	52.49	54.26	1.77
GN *	2013	16864.52	16877.15	12.63
	2014	20061.94	20840.71	778.77



Env	Background	Chromosome	GY	TGW	GN
2013	Avalon	1B			
		1D			
		2A			
		2D			
		3A			+
		3B		-	
		5A		+	
		6A		-	
		6B			
		7B			-
		7D			
	Cadenza	1B			
		1D			
		2A			
		2D			
		3A	+		+
		3B			
		6A		-	
		6B			
2014	Avalon	1B	+		
		1D	-		-
		2A			
		2D			
		3A			
		3B			
		5A		+	
		6A		-	
		6B			
		7B	-		-
		7D			
	Cadenza	1B			
		1D			
		2A			
		2D			
		3A	+		+
		3B			
		6A		-	+
		6B			

- = Avalon allele ↑
+ = Cadenza allele ↑

Env	Background	Chromosome	GY	TGW	Area	Length	Width	L/W	GN
2013	Avalon	1B							
		1D							
		2A							
		2D							
		3A				🟥 -			🟥 +
		3B		🟦 -		🟦 -		🟦 -	
		5A		🟦 +	🟦 +	🟦 +	🟦 +		
		6A		🟦 -	🟦 -		🟦 -		
		6B							
		7B			🟨 +		🟨 +	🟨 -	🟨 -
		7D							
	Cadenza	1B							
		1D							
		2A							
		2D						+	
		3A	🟥 +			🟥 -		🟥 -	🟥 +
		3B							
		6A		🟦 -	🟦 -	🟦 -	🟦 -		
		6B							
2014	Avalon	1B	🟥 +						
		1D	🟥 -						🟥 -
		2A				+			
		2D							
		3A						🟥 -	
		3B							
		5A		🟦 +	🟦 +	🟦 +	🟦 +		
		6A		🟦 -	🟦 -		🟦 -		
		6B							
		7B	🟥 -						🟥 -
		7D							
	Cadenza	1B							
		1D							
		2A							
		2D							
		3A	🟥 +			🟥 -		🟥 -	🟥 +
		3B							
		6A		🟦 -	🟦 -		🟦 -		🟦 +
		6B							

neutral effects for PH and HD penalty

- = Avalon allele ↑
+ = Cadenza allele ↑

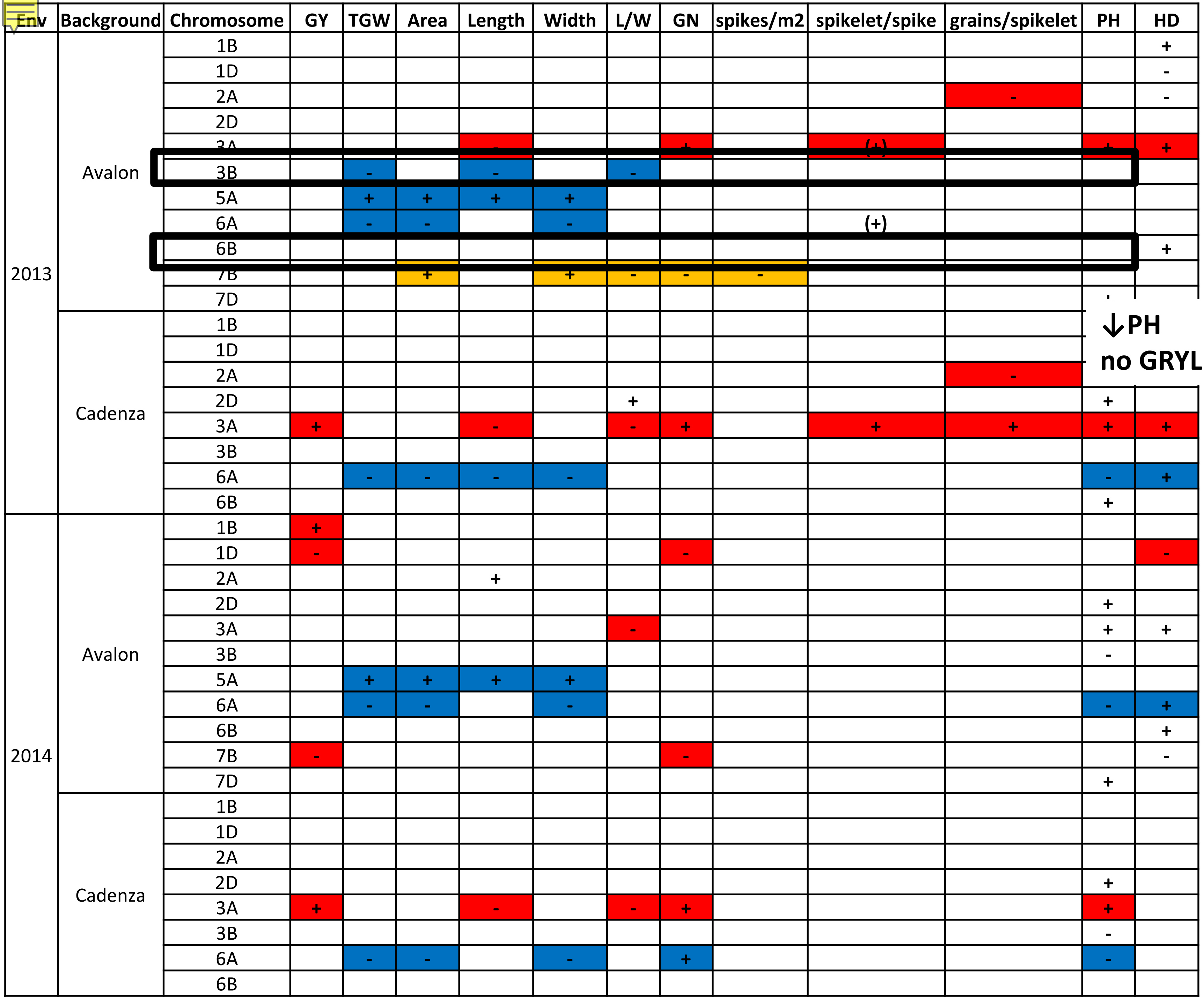
Env	Background	Chromosome	GY	TGW	Area	Length	Width	L/W	GN
2013	Avalon	1B							
		1D							
		2A							
		2D							
		3A				-			+
		3B		-		-		-	
		5A		+	+	+	+		
		6A		-	-		-		
		6B							
		7B			+		+	-	-
		7D							
	Cadenza	1B							
		1D							
		2A							
		2D						+	
		3A	+			-		-	+
		3B							
		6A		-	-	-	-		
		6B							
2014	Avalon	1B	+						
		1D	-						-
		2A				+			
		2D							
		3A						-	
		3B							
		5A		+	+	+	+		
		6A		-	-		-		
		6B							
		7B	-						-
		7D							
	Cadenza	1B							
		1D							
		2A							
		2D							
		3A	+			-		-	+
		3B							
		6A		-	-		-		+
		6B							

PH penalty

- = Avalon allele ↑
+ = Cadenza allele ↑

Env	Background	Chromosome	GY	TGW	Area	Length	Width	L/W	GN	spikes/m2	spikelet/spike	grains/spikelet
2013	Avalon	1B										
		1D										
		2A										🟥 -
		2D										
		3A				🟥 -			🟥 +		🟥 (+)	
		3B		🟦 -		🟦 -		🟦 -				
		5A		🟦 +	🟦 +	🟦 +	🟦 +					
		6A		🟦 -	🟦 -		🟦 -				(+)	
		6B										
		7B			🟧 +		🟧 +	🟧 -	🟧 -	🟧 -		
		7D										
	Cadenza	1B										
		1D										
		2A										🟥 -
		2D						+				
		3A	🟥 +			🟥 -		🟥 -	🟥 +		🟥 +	🟥 +
		3B										
		6A		🟦 -	🟦 -	🟦 -	🟦 -					
		6B										
2014	Avalon	1B	🟥 +									
		1D	🟥 -						🟥 -			
		2A				+						
		2D										
		3A						🟥 -				
		3B										
		5A		🟦 +	🟦 +	🟦 +	🟦 +					
		6A		🟦 -	🟦 -		🟦 -					
		6B										
		7B	🟥 -						🟥 -			
		7D										
	Cadenza	1B										
		1D										
		2A										
		2D										
		3A	🟥 +			🟥 -		🟥 -	🟥 +			
		3B										
		6A		🟦 -	🟦 -		🟦 -		🟦 +			
		6B										

- = Avalon allele ↑
+ = Cadenza allele ↑



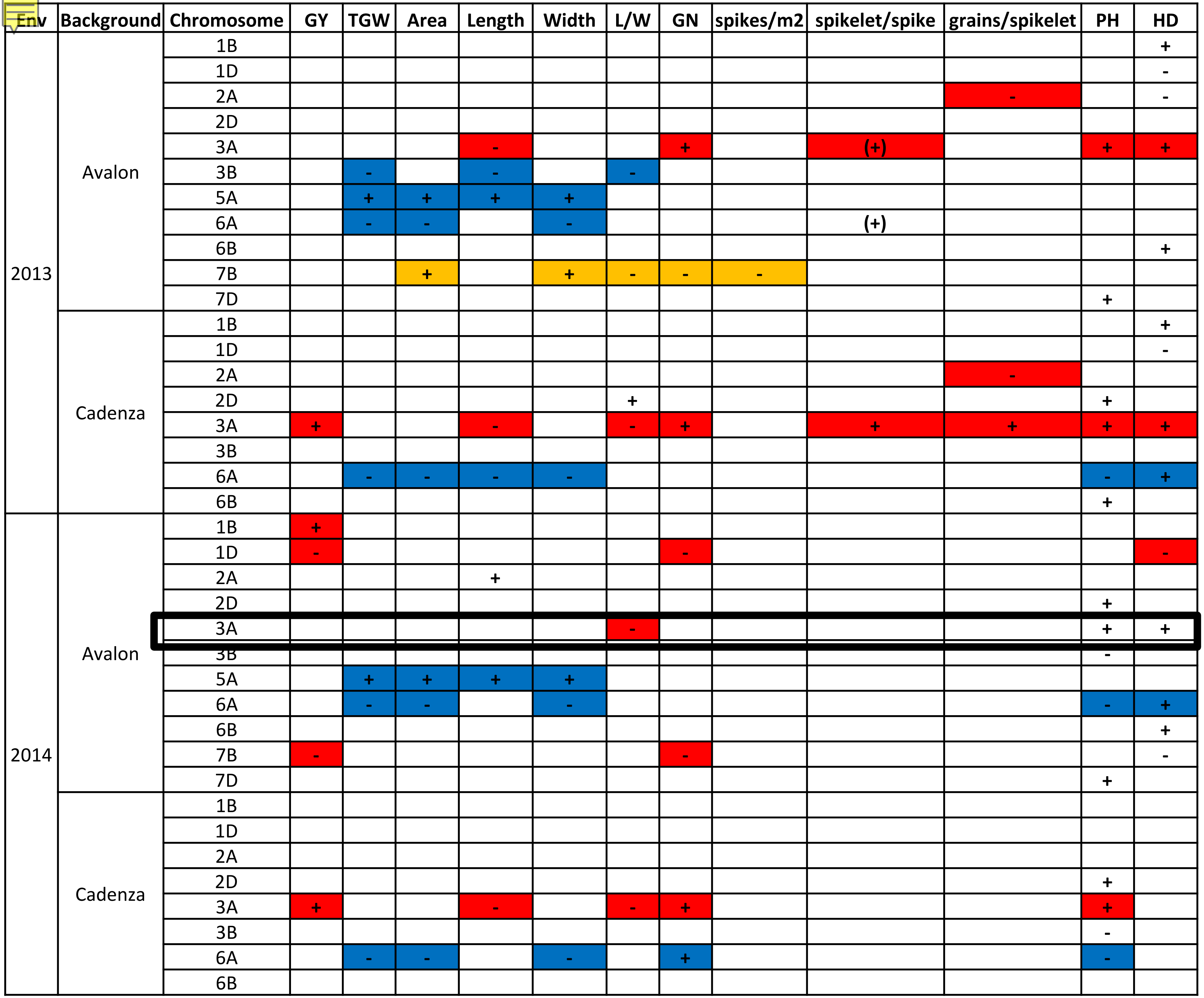
Env	Background	Chromosome	GY	TGW	Area	Length	Width	L/W	GN	spikes/m2	spikelet/spike	grains/spikelet	PH	HD
2013	Avalon	1B												+
		1D												-
		2A										-		-
		2D												
		3A				-			+		(+)		+	+
		3B		-		-		-						
		5A		+	+	+	+							
		6A		-	-		-				(+)			
		6B												+
		7B			+		+	-	-	-				
		7D											+	
	Cadenza	1B												
		1D												
		2A										-		
		2D						+					+	
		3A	+			-		-	+		+	+	+	+
		3B												
		6A		-	-	-	-						-	+
		6B											+	
2014	Avalon	1B	+											
		1D	-						-					-
		2A				+								
		2D											+	
		3A						-					+	+
		3B											-	
		5A		+	+	+	+							
		6A		-	-		-						-	+
		6B												+
		7B	-						-					-
		7D											+	
	Cadenza	1B												
		1D												
		2A												
		2D											+	
		3A	+			-		-	+				+	
		3B											-	
		6A		-	-		-		+				-	
		6B												

↓PH

no GRYLD penalty

- = Avalon ↑

+ = Cadenza ↑



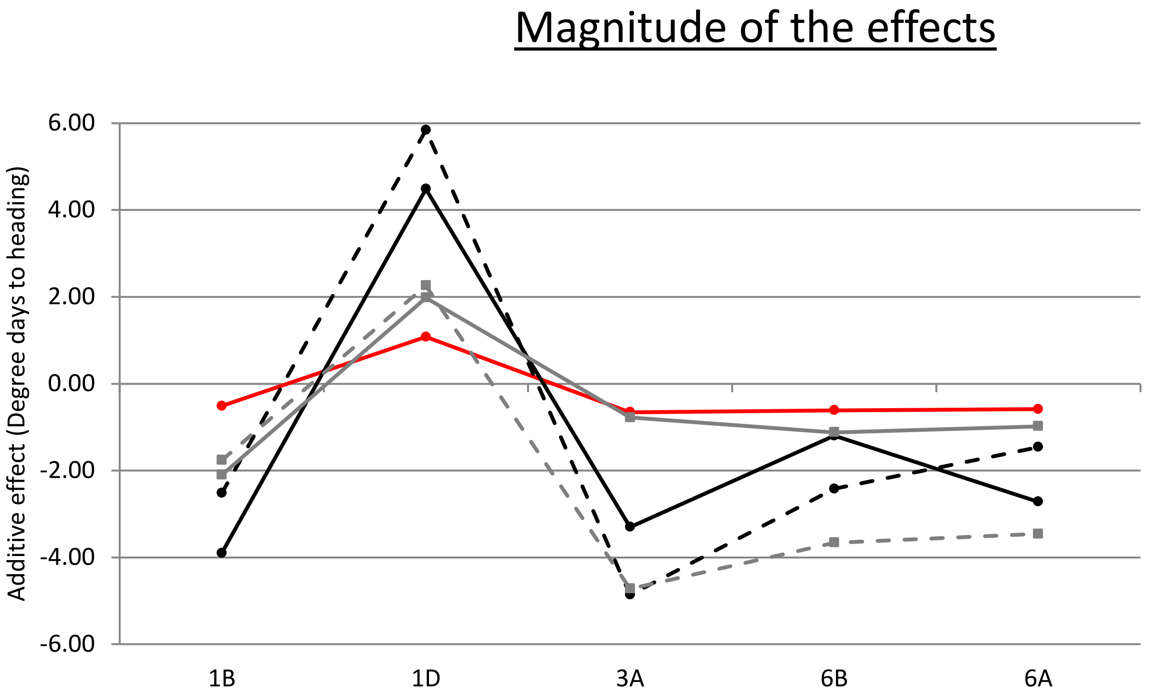
Env	Background	Chromosome	GY	TGW	Area	Length	Width	L/W	GN	spikes/m2	spikelet/spike	grains/spikelet	PH	HD
2013	Avalon	1B												+
		1D												-
		2A										-		-
		2D												
		3A				-			+		(+)		+	+
		3B		-		-		-						
		5A		+	+	+	+							
		6A		-	-		-				(+)			
		6B												+
		7B			+		+	-	-	-				
		7D											+	
	Cadenza	1B												+
		1D												-
		2A										-		
		2D						+					+	
		3A	+			-		-	+		+	+	+	+
		3B												
		6A		-	-	-	-						-	+
		6B											+	
2014	Avalon	1B	+											
		1D	-						-					-
		2A				+								
		2D											+	
		3A						-					+	+
		3B											-	
		5A		+	+	+	+							
		6A		-	-		-						-	+
		6B												+
		7B	-						-					-
		7D											+	
	Cadenza	1B												
		1D												
		2A												
		2D											+	
		3A	+			-		-	+				+	
		3B											-	
		6A		-	-		-		+				-	
		6B												

= TGRWT

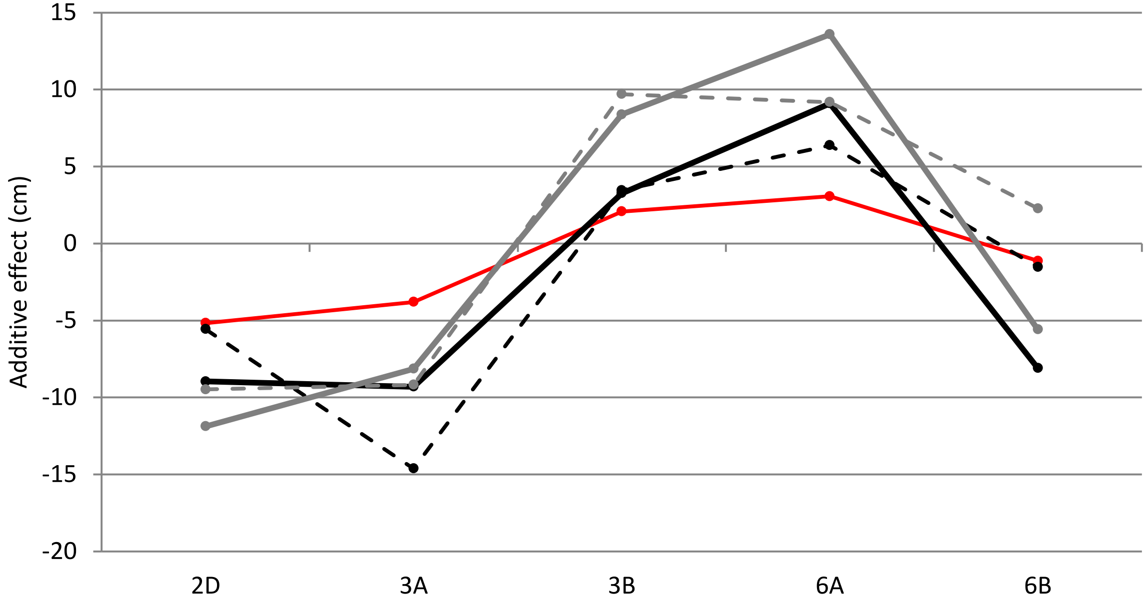
\- = Avalon ↑
\+ = Cadenza ↑

Magnitude of the effects

HD



PH

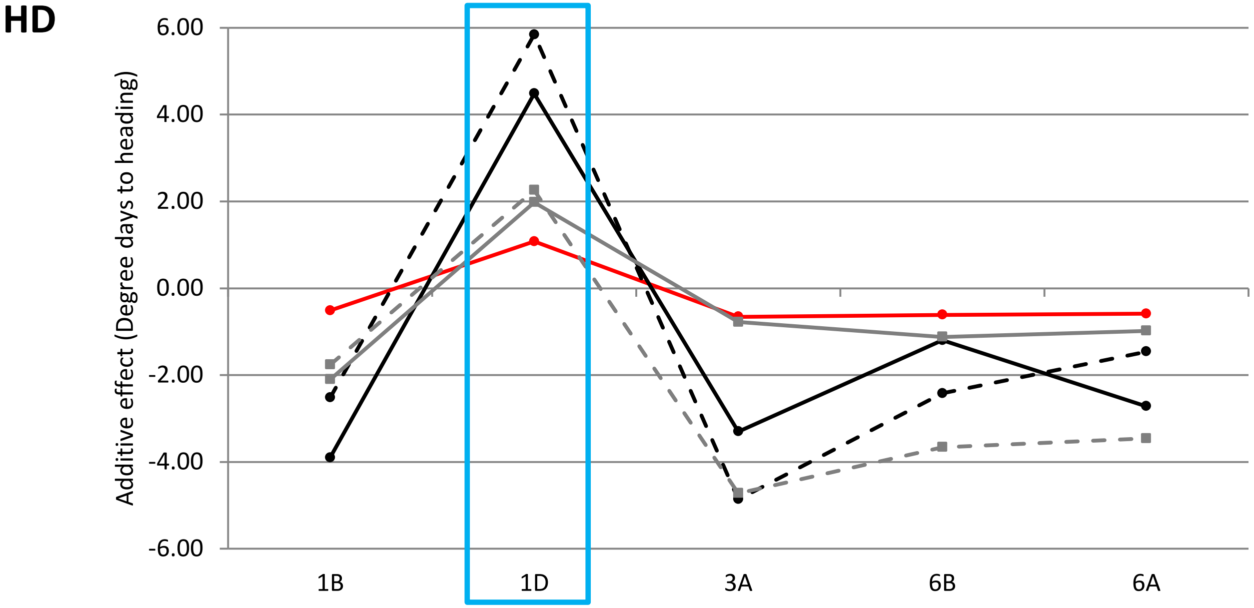


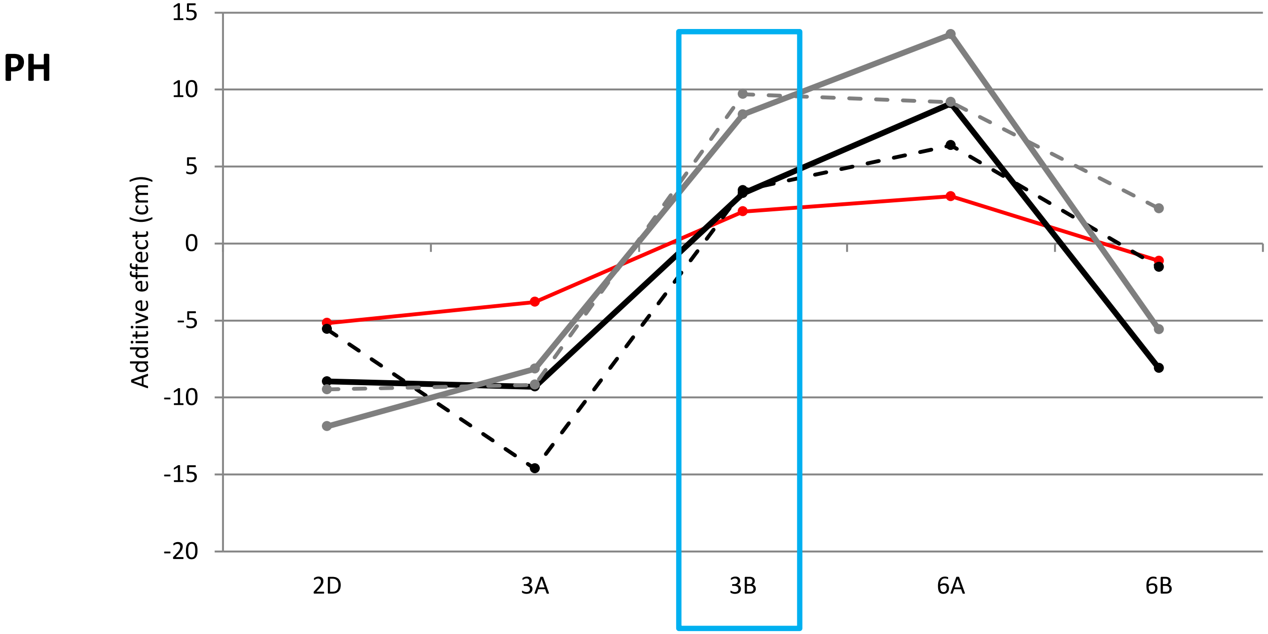
QTL×E interaction

QTL× Background interaction

weak phenotypic effects

Magnitude of the effects

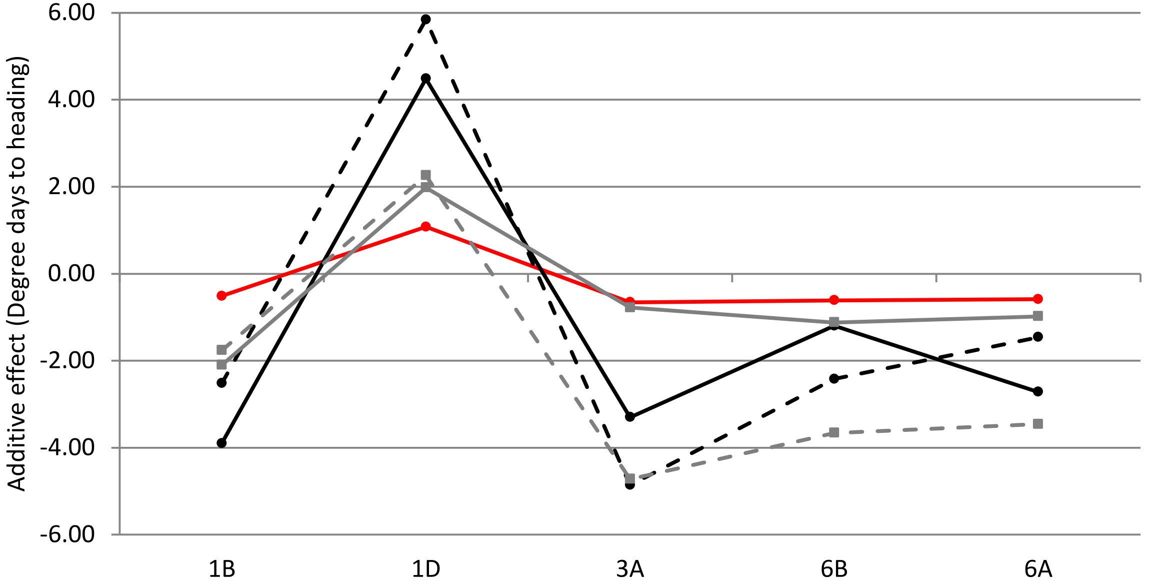




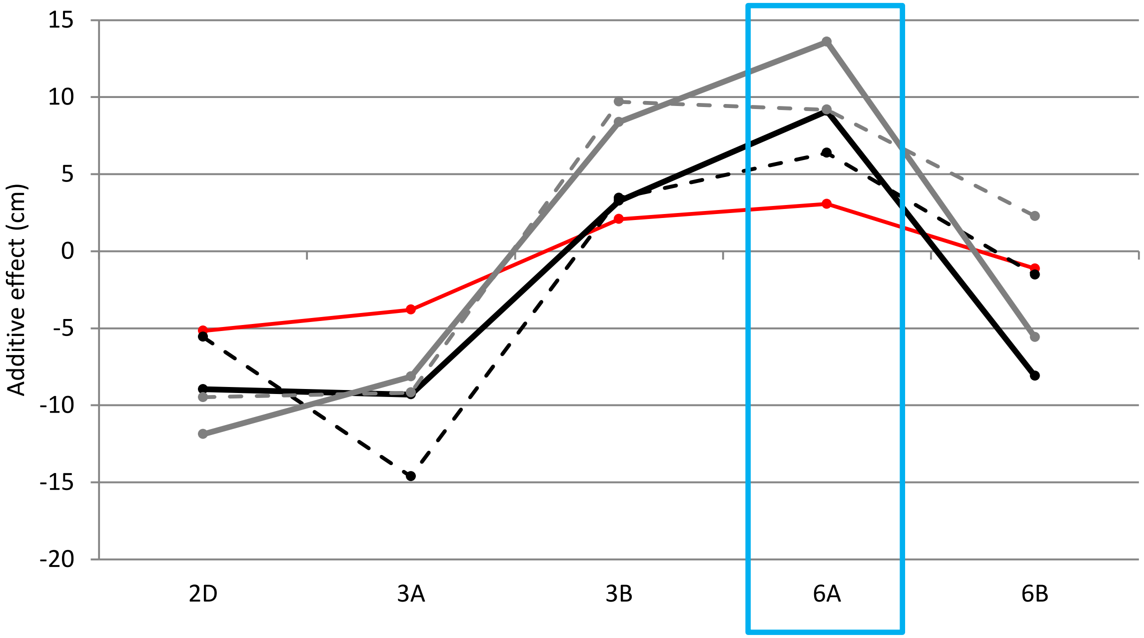
QTL×E interaction
QTL× Background interaction
weak phenotypic effects

Magnitude of the effects

HD



PH

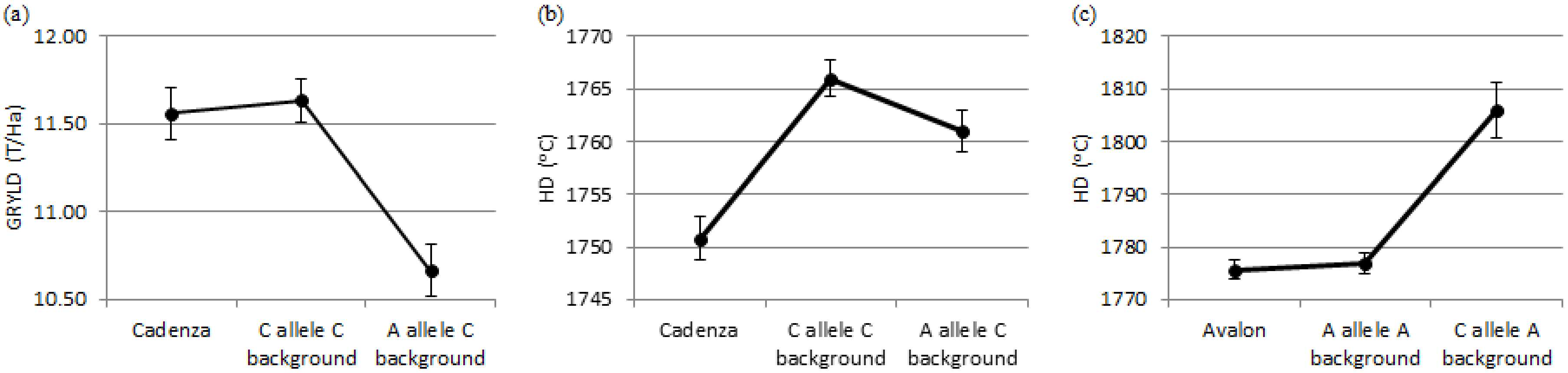


QTL×E interaction

QTL× Background interaction

weak phenotypic effects

Possible background effects (i.e. 3A)



(A = Avalon; C = Cadenza)

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WGIN Meeting

Clare Lister

17/7/2015

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 - **ALBA**
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

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WGIN3 Projects: Griffiths Lab

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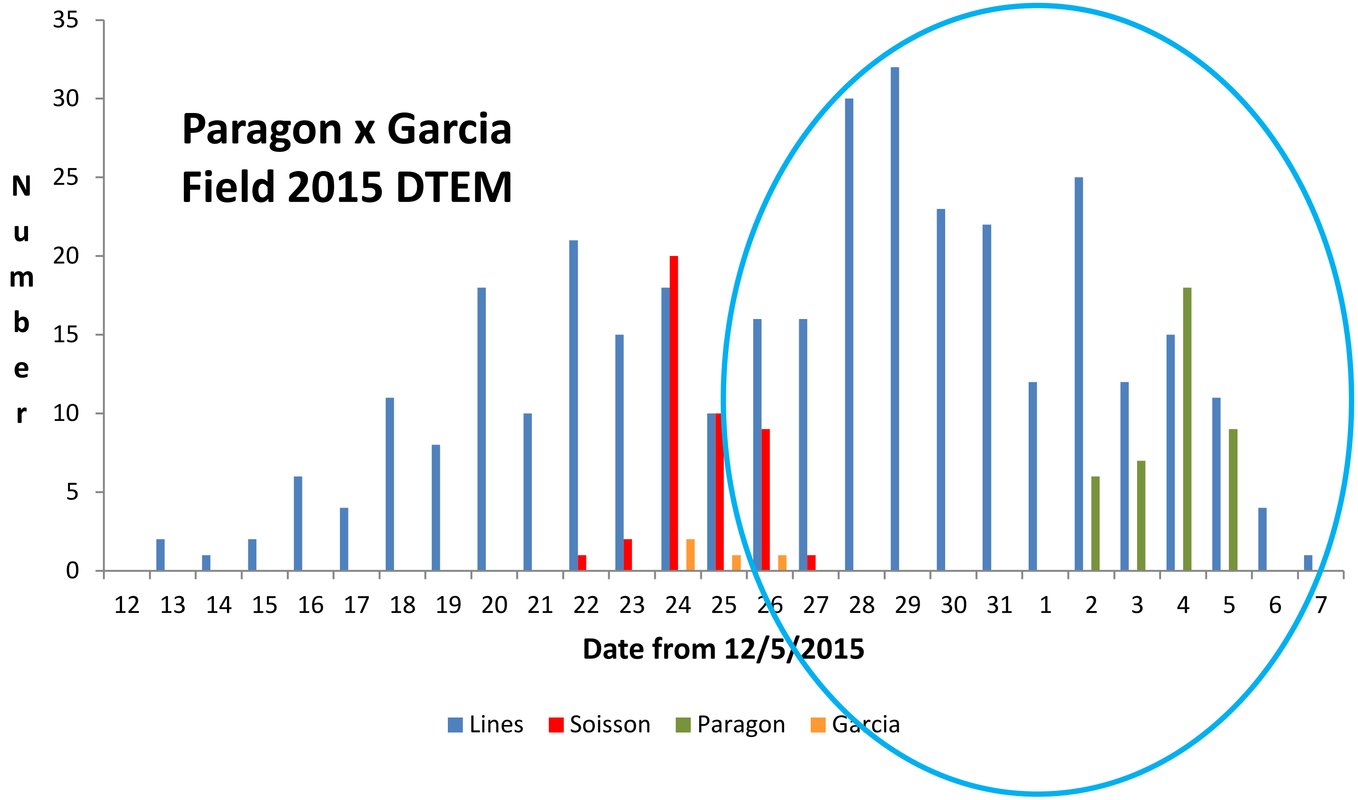
1. Dissecting UK drought tolerance in Paragon x Garcia

- RIL’s scored for DTEM and Height
- Yield to be measured
- Drought Trial planned for 2015-2016 with selected lines



WGIN3 Projects: Griffiths' Lab

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

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

- 3 rep, spring-sown, yield-trial of subset of Paragon Library
- DTEM scored
- Height and yield to be measured

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WGIN3 Projects: Griffiths' Lab

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

- NIL stacking i.e. Rht1 x Rht8 –> **F2 seed**

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

- Creating as many F1's for future development of linked populations targeting UK yield stability

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

- Following on from theoretical work in Ma *et al* (2015)* – crosses made between three ideal NILs

(Work by Simon Orford, to be continued by CL)

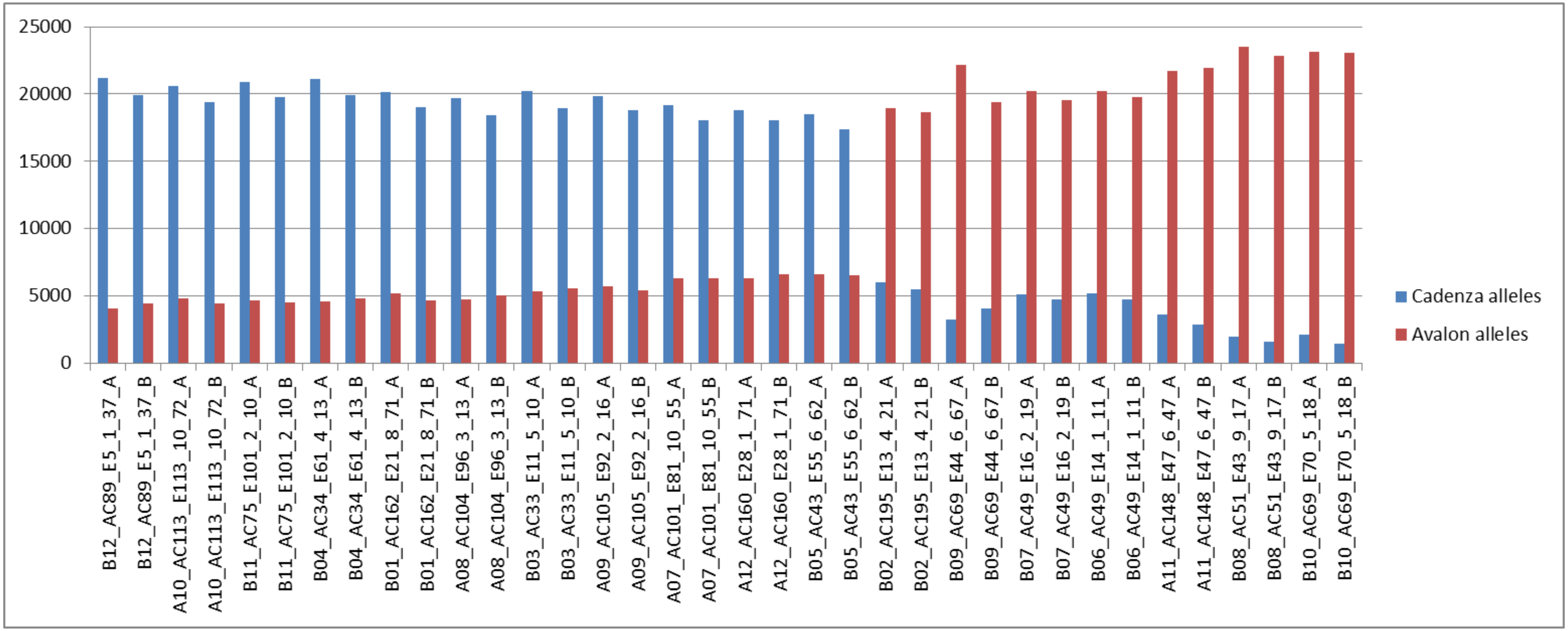
*"Using the UK reference population Avalon × Cadenza as a platform to compare breeding strategies in elite Western European bread wheat" *Molecular Breeding* 35

WGIN3 Projects: Griffiths' Lab

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

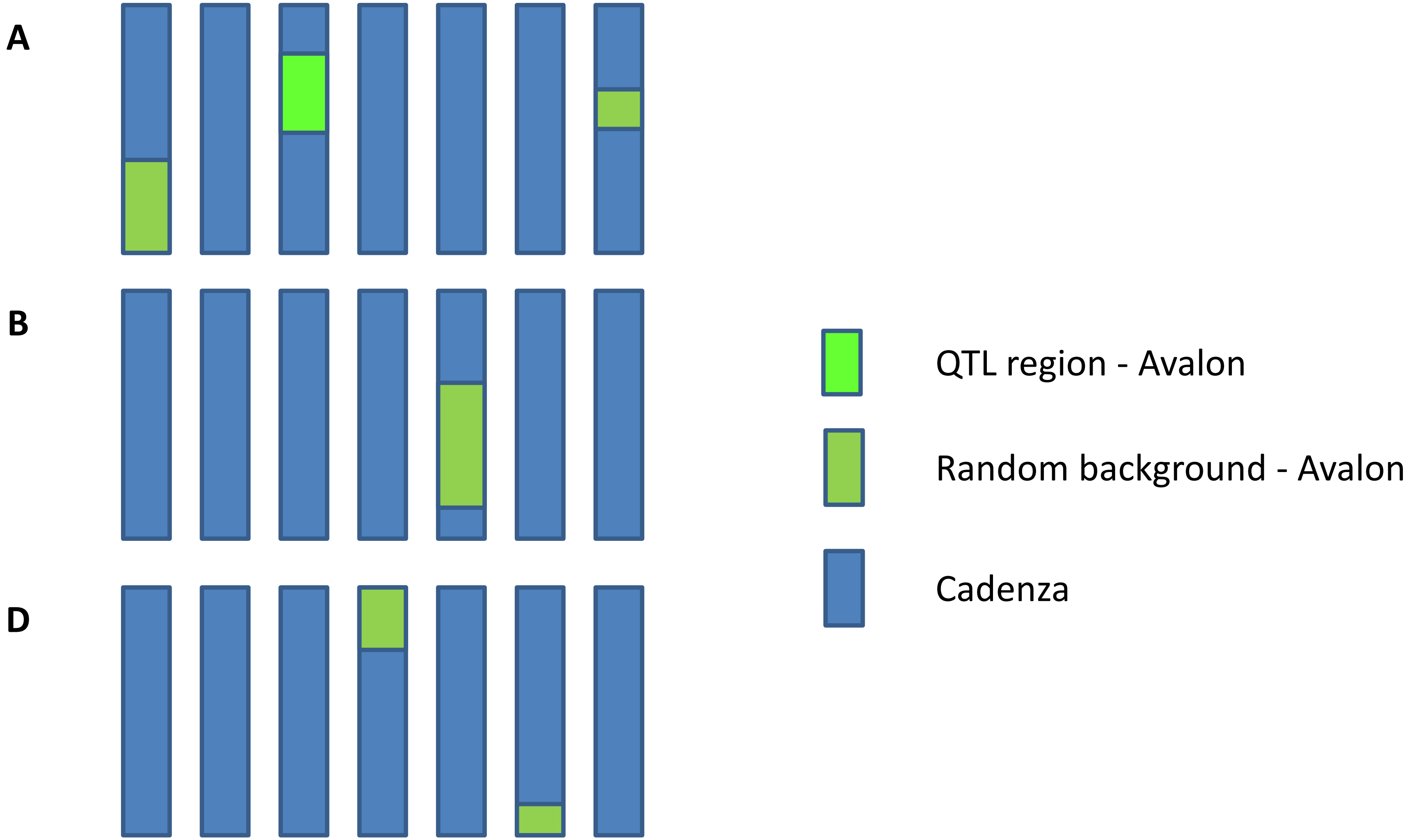
- WGIN successfully promoted the A x C DH population as UK reference population
- A x C population most densely mapped in the world
- Much phenotypic data also available
- NILs derived from these have validated QTLs

- The BC3 NILs carry selected genetic foreground in the QTL regions (height, heading, and yield)
- In addition each line carries ~12.5% random chromosomal regions.



18 NILs genotyped on the 820K array

QTL region and random background



This could also allow an understanding of the interactions between the specific QTL and other regions of the genome, which may, or may not be, other known QTL loci.

A CSSL for Avalon x Cadenza

- 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?

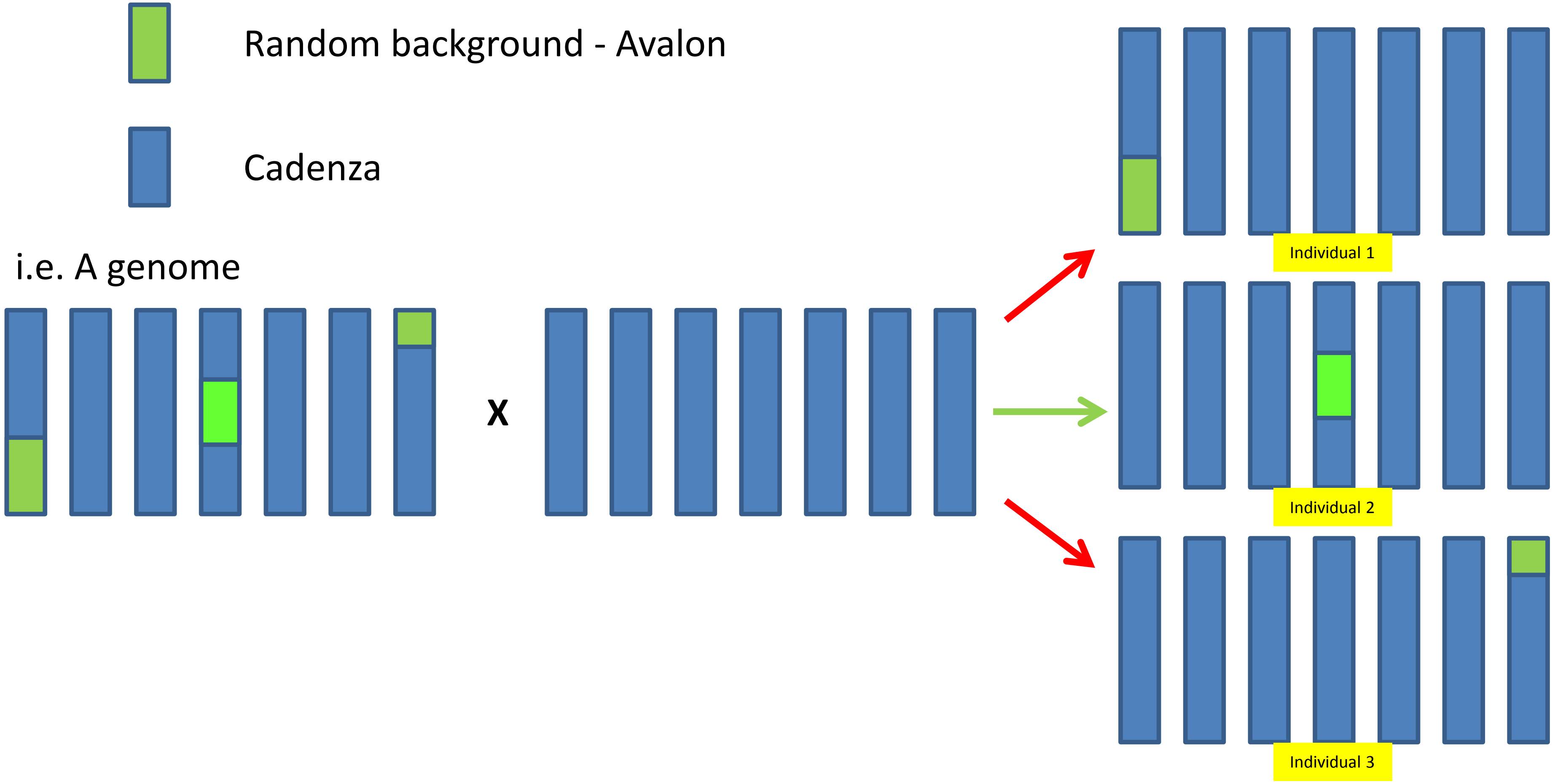
- 250 BC_2 NILs in Avalon background
- 302 BC_2 NILs in Cadenza background

🟩 QTL region - Avalon

🟩 Random background - Avalon

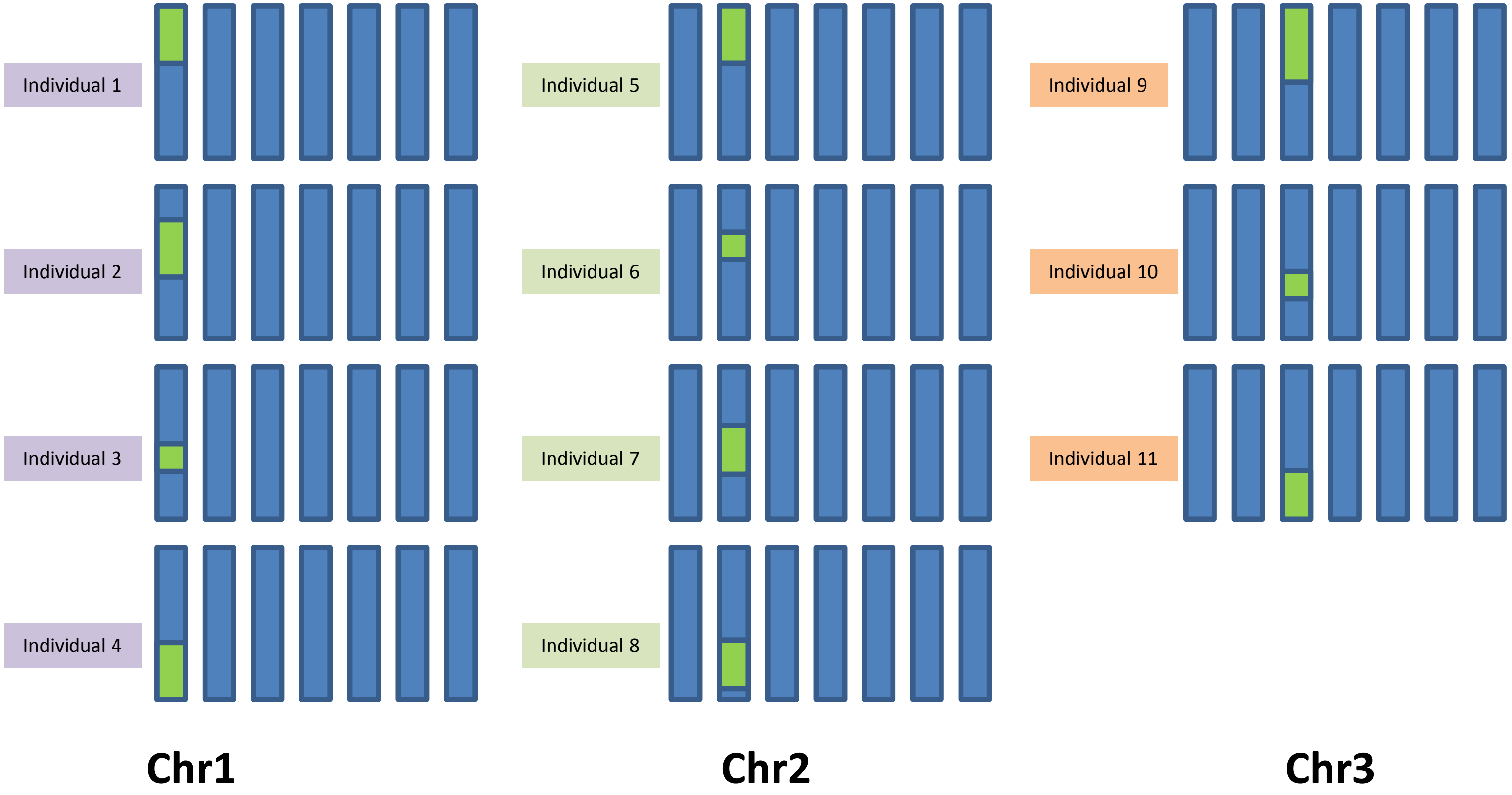
🟦 Cadenza

i.e. A genome



Simplistic (and optimistic) representation!

i.e. A genome, chromosomes 1, 2 and 3 ……



Selection of lines for CSSL

- 47 Avalon+b allele and 47 Cadenza+a allele lines = **94 lines**
- representing all the 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

- Selection also based on various data
  - Previous genotyping to determine background
  - Lines where backcrosses already made
  - Lines at the extremes of the QTL phenotypic data

Requirements for CSSL

- Need maps for chosen NILs
- Full AxC Map (18 942 markers) from Bristol
- Frame AxC Map (1 286 markers) from Bristol
- Already have 820K Axiom data for 18 NILs
- Genotyping of 94 lines on 35K Axiom array

- Need markers in 820K array also in 35K for maps
- Preferably use markers in Frame Map – not always possible
- Preferably scored as AA/BB – reduces genotype ambiguities
- Preferably are BS markers – useful for small-scale genotypers

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i.e. Chromosome 3A

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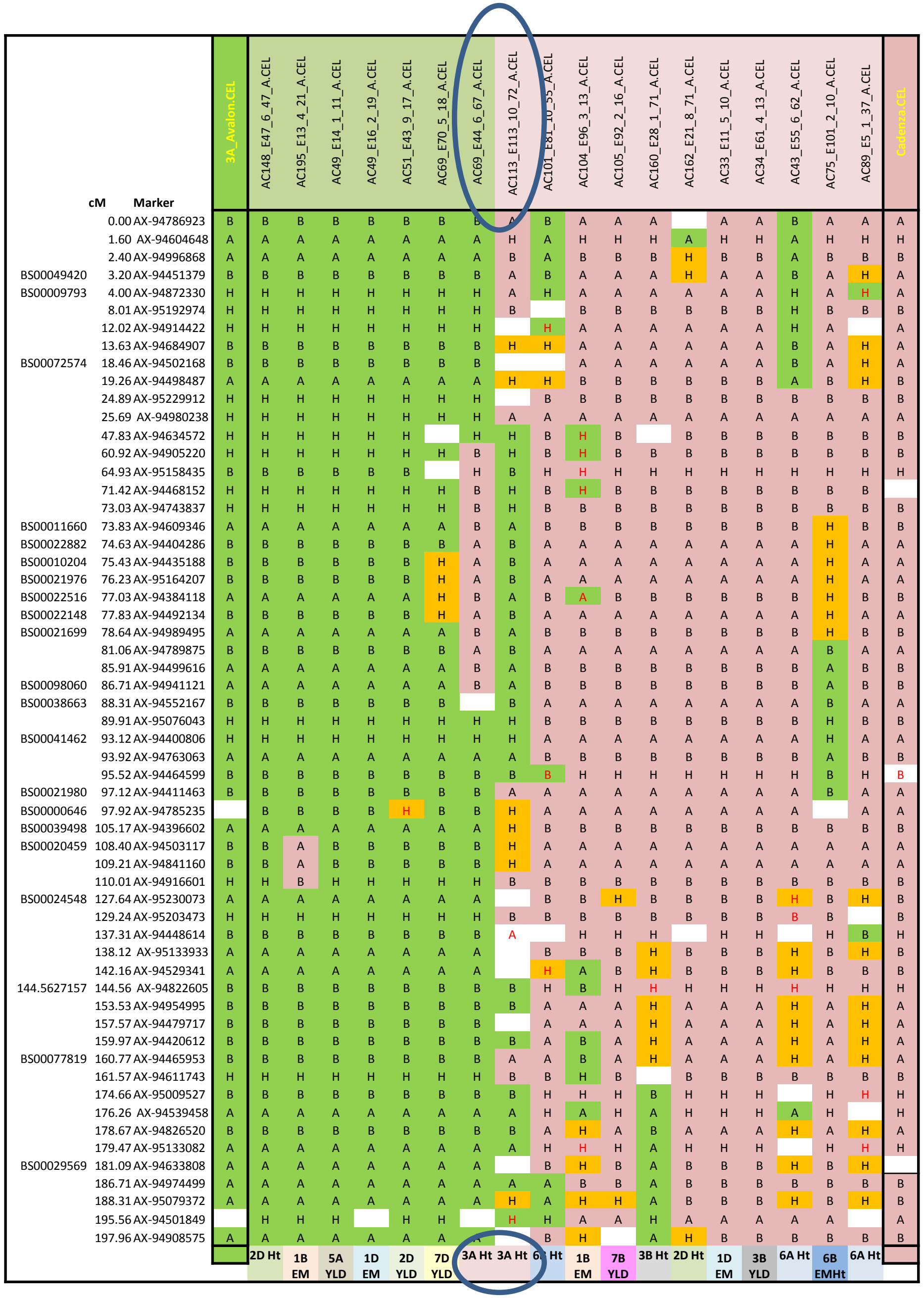

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	1.60	AX-94604648	A	A	A	A	A	A	A	A	H	A	H	H	H	A	H	H	A	H	H	H
	2.40	AX-94996868	A	A	A	A	A	A	A	A	B	A	B	B	B	H	B	B	A	B	B	B
BS00049420	3.20	AX-94451379	B	B	B	B	B	B	B	B	A	B	A	A	A	H	A	A	B	A	H	A
BS00009793	4.00	AX-94872330	H	H	H	H	H	H	H	H	A	H	A	A	A	A	A	A	H	A	H	A
	8.01	AX-95192974	H	H	H	H	H	H	H	H	B		B	B	B	B	B	B	H	B	B	B
	12.02	AX-94914422	H	H	H	H	H	H	H	H		H	A	A	A	A	A	A	H	A		A
	13.63	AX-94684907	B	B	B	B	B	B	B	B	H	H	A	A	A	A	A	A	B	A	H	A
BS00072574	18.46	AX-94502168	B	B	B	B	B	B	B	B			A	A	A	A	A	A	B	A	H	A
	19.26	AX-94498487	A	A	A	A	A	A	A	A	H	H	B	B	B	B	B	B	A	B	H	B
	24.89	AX-95229912	H	H	H	H	H	H	H	H		B	B	B	B	B	B	B	B	B	B	B
	25.69	AX-94980238	H	H	H	H	H	H	H	H	A	A	A	A	A	A	A	A	A	A	A	A
	47.83	AX-94634572	H	H	H	H	H	H		H	H	B	H	B		B	B	B	B	B	B	B
	60.92	AX-94905220	H	H	H	H	H	H	H	B	H	B	H	B	B	B	B	B	B	B	B	B
	64.93	AX-95158435	B	B	B	B	B	B		H	B	H	H	H	H	H	H	H	H	H	H	H
	71.42	AX-94468152	H	H	H	H	H	H	H	B	H	B	H	B	B	B	B	B	B	B	B	
	73.03	AX-94743837	H	H	H	H	H	H	H	B	H	B	B	B	B	B	B	B	B	B	B	B
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BS00022882	74.63	AX-94404286	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A	A	H	A	A
BS00010204	75.43	AX-94435188	B	B	B	B	B	B	H	A	B	A	A	A	A	A	A	A	A	H	A	A
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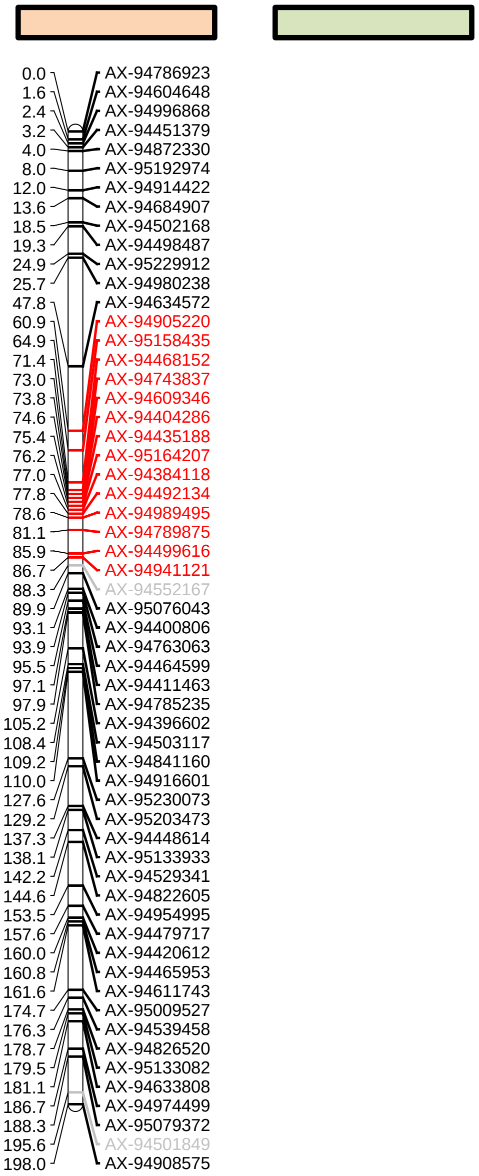
	179.47	AX-95133082	A	A	A	A	A	A	A	A	A	H	H	H	A	H	H	H		H	H	H
BS00029569	181.09	AX-94633808	A	A	A	A	A	A	A	A		B	H	B	A	B	B	B	H	B	H	
	186.71	AX-94974499	A	A	A	A	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B
	188.31	AX-95079372	A	A	A	A	A	A	A	A	H	A	H	H	A	B	B	B	H	B	H	B
	195.56	AX-94501849		H	H	H		H	H		H	H	A	A	H	A	A	A	A	A		A
	197.96	AX-94908575	A	A	A	A	A	A	A	A		B	H		A	H	B	B	B	B	B	B
				2D Ht	1B EM	5A YLD	1D EM	2D YLD	7D YLD	3A Ht	3A Ht	6[illegible] Ht	1B EM	7B YLD	3B Ht	2D Ht	1D EM	3B YLD	6A Ht	6B EMHt	6A Ht	

Maps of Chromosome 3

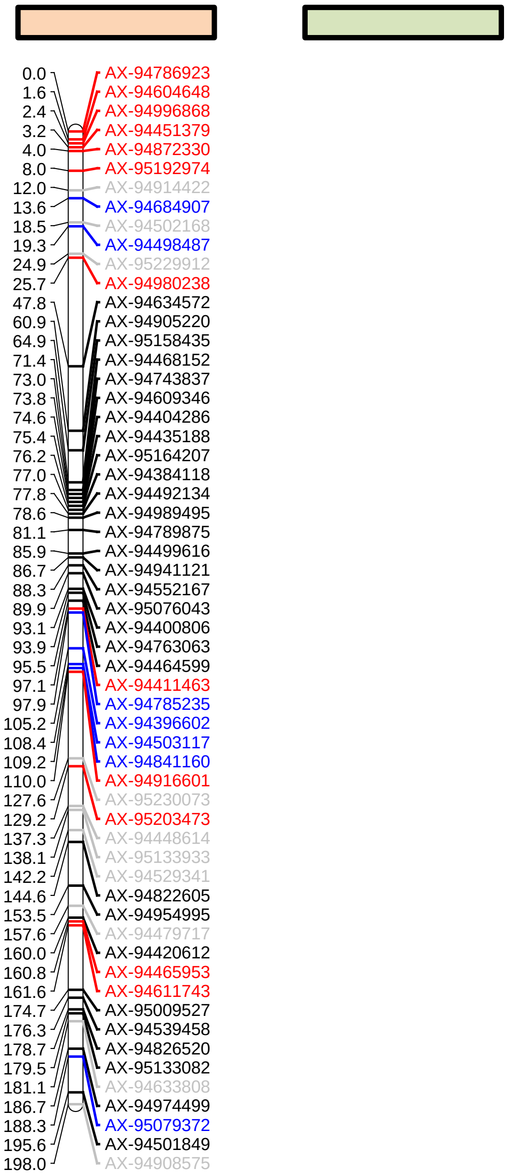
AC69_E44_6_67_All

3A Ht in Avalon background



AC113_E113_10_72_All

3A Ht in Cadenza background



Maps from 35K and 820K
AxC array data,
plus additional markers from
Frame Map not in 35K array
(**18 maps -> WGIN website**)

Maps from 35K and 820K
AxC array data only
(**94 maps**)

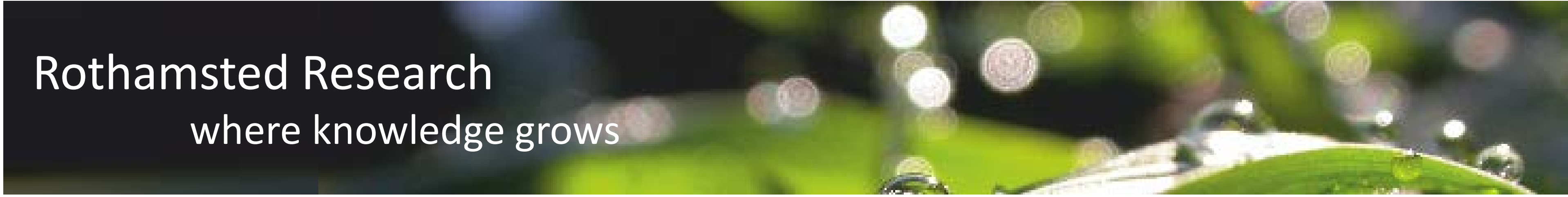
Avalon
Cadenza
Het
No marker data

Almost ready to genotype…..!

- Leaf material harvested, DNA preps next week!
- -> Genotyping on 35K Axion wheat breeders array
- Make maps of all 94 lines -> WGIN website

Subsequent work…

- Backcross lines twice to recurrent parent
- KASP markers will be used to select for the new target segment.
- Lines selfed and homozygous CSSLs selected
- Lines available for use



Rothamsted Research

where knowledge grows

WGIN 3

Andrew B Riche

2nd Management Meeting
17th July 2015

defra
Department for Environment Food and Rural Affairs

Wheat
Genetic
Improvement
Network

BBSRC
bioscience for the future

ROTHAMSTED RESEARCH

26/6/15

Wheat varieties for WGIN 20:20-NUE
2015/16 W=WGIN data, D=desk study

Variety	Source	Nabim	Rationale	Previous years of trials (harvest year)
1. Avalon		1	WGIN DH parent; Low NupE & NutE (D) WUE trial	05-15
2. Bonham	KWS	2?	Low TAB parentage W104 (Portland) x Cordiale	14-15
3. Cadenza		2	WGIN DH parent; Best NupE (W) WUE trial	04-15
4. Claire	LIM	3	Was biggest area on RL; WGIN DH parent; Good second wheat	05-15
5. Cocoon	Agrii/Secobra	3	Tall variety. High yield. 2010 introduction. Eyespot and rust resistant.	13-15
6. Conqueror	KWS	4	New Grp 4, very high yielding	12-15
7. Cordiale	KWS	2	Good second wheat. BBSRC Quality project WUE trial	06-15
8. Crusoe	LIM	2	Carries dicoccoides. Shows the 'stay green' character	11-15
9. Evoke	KWS	2?	Low TAB? Cordiale x W134 Timaru	14-15
10. Gallant	Syn	1	new claimed high yield and high protein type	10-15
11. Hereford	Syn	4	Feed (not on RL), high yield, brown rust susceptible, possible low take-all build-up and good resistance. Multi trait.	12-15
12. Hereward	RAGT	1	Best protein on RL; benchmark bread variety. BBSRC Quality project WUE trial	04-15
13. Hystar	Saaten Union	4	Hybrid for the first time, soft feed, high yield, good roots	15
14. Istabraq	LIM	4	Best yield on RL; Distilling cultivar; In LINK 'GREENgrain'; Good second wheat. BBSRC Quality project. WUE trial	05-15
15 Malacca	KWS	1	Biggest Group 1 area; DH choice; Low NupE, high NutE (W). BBSRC Quality project	04-15
16. Maris Widgeon		1	Tall (rht), old cultivar WUE trial	04-15
17. Mercia		1	Low NupE & NutE (desk); Low Canopy N requirement; In IGF micro-array. WUE trial. RHT series	04, 06-15
18. Paragon	RAGT	1	Spring variety; WGIN mutagenesis population; High NupE (W)	04-15
19. Riband	RAGT	3	WGIN DH parent; Distilling cultivar; In LINK 'GREENgrain'; High NutE (W)	04-15
20. Robigus	KWS	3	Best Group 3 yield; Best NUE, high NupE & NutE (D); Good second wheat. WUE trial	05-15
21. Skyfall	RAGT	1	Still provisional RL as of June 2014 but very high yielding Grp 1	15
22. Stigg	LIM	?4	Carries dicoccoides. High disease resistance. Shows the 'stay green' character	11-15
23. Soissons	Elsoms	2	WGIN DH parent; Early maturing; High NupE, low NutE (W) WUE trial	04-15
24. Solstice	LIM	2	Biggest Group 2 area; DH choice; Worst NupE (W)	04-15
25. Xi19	LIM	1	Best Group 1 yield; High NUE, NupE, NutE (D); Low NupE (W). BBSRC Quality project. WUE trial	04-15

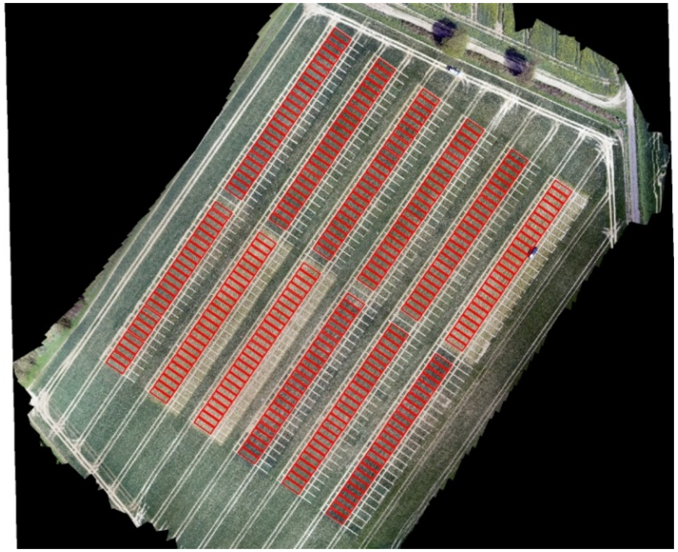
Wheat varieties for WGIN 20:20-NUE
2015/16

Variety	Source	Nabim	Rationale
26. Evolution	**Limagrain**	**4**	**High yielding. Hard wheat. Consistent? Moderate straw length.**
27. KWS Lili	**KWS**	**2**	**Very high yield.. Short and stiff straw,.**
28. Reflection	**Syngenta**	**4**	**Early maturing. High yielding hard milling.**
29. RGT Illustrious	**RAGT**	**cand**	**Candidate for 2016/17. For breadmaking. Good quality and breadmaking ability even with low protein**
30. Hylux	**Saaten Union**		**Hybrid. Early flowering and maturing. Can be mildew susceptible; treat T0. Good under stress? Breadmaking?**

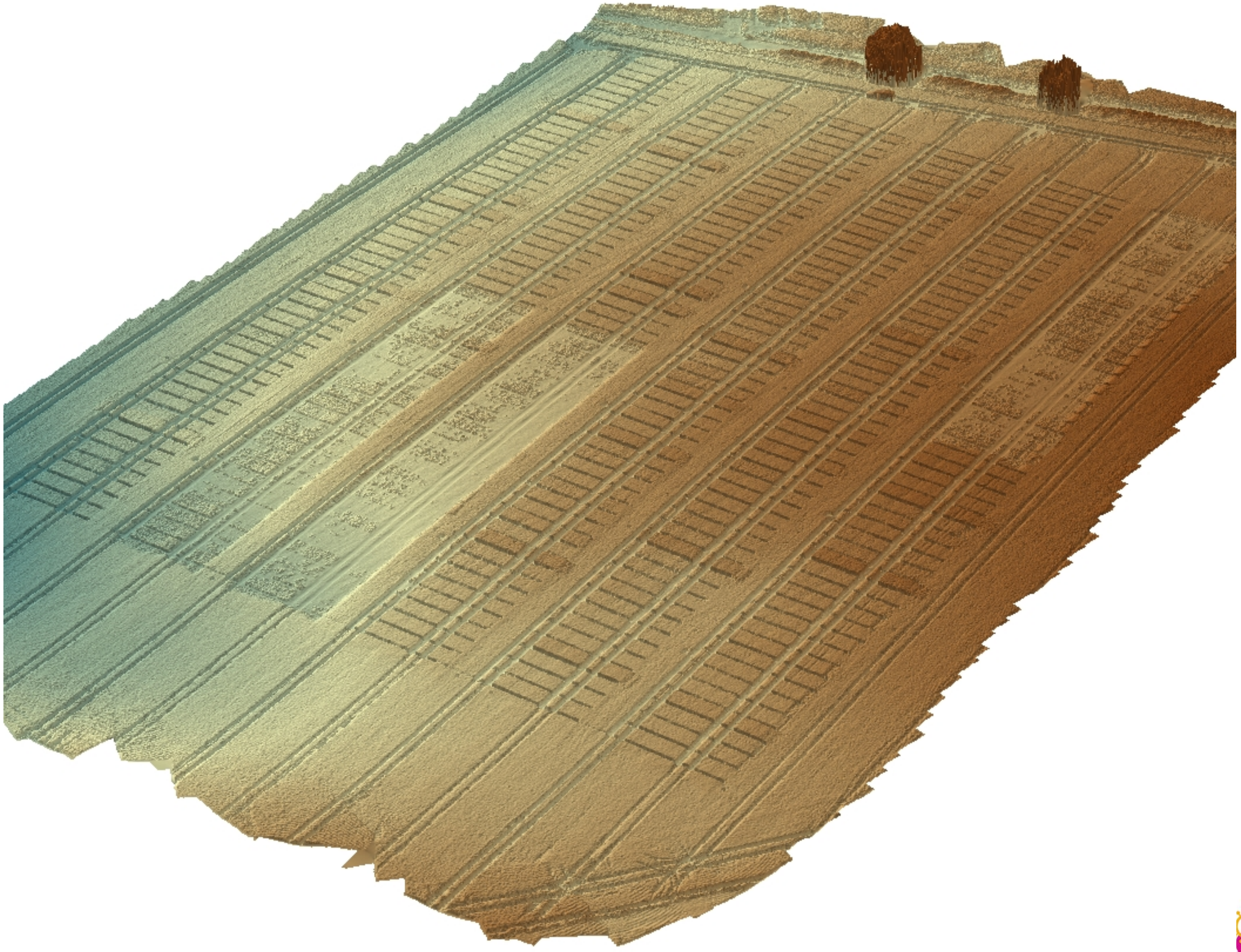
Aerial imaging

Orthomosaic photo requirements:

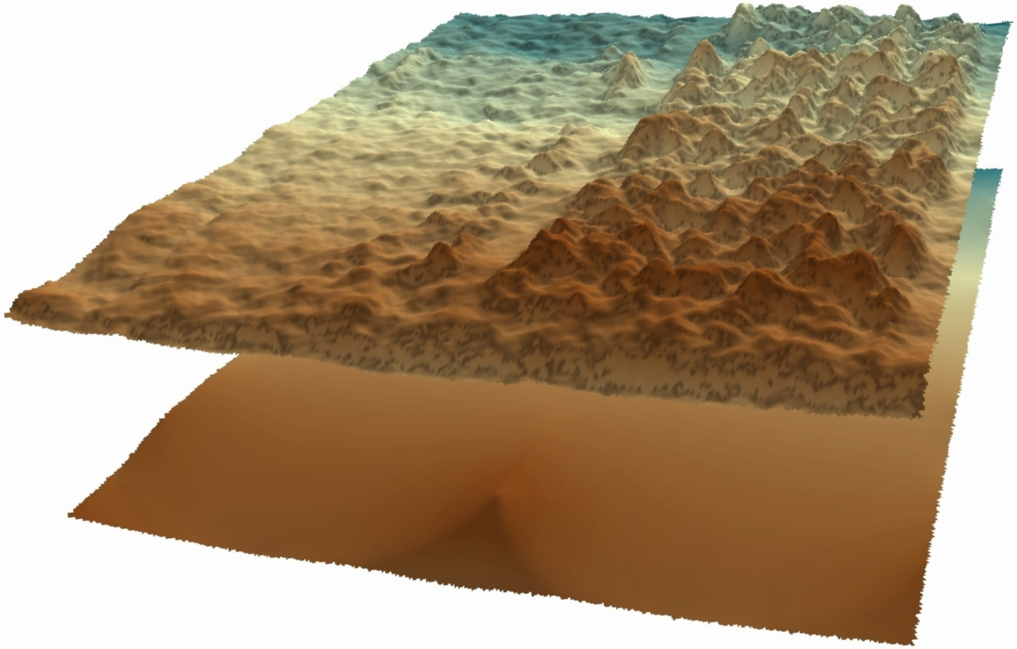
- Typically 500 photos per expt
- 80% overlap
- 12 GCPs per experiment
- Can take >24hrs to process images



Create DEM/DSM

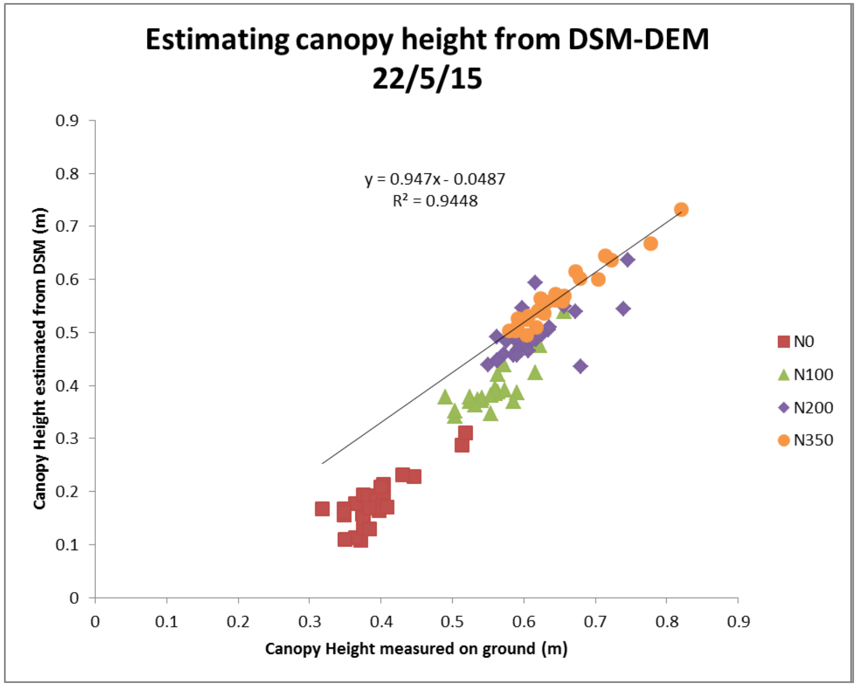


Subtracting ground variation



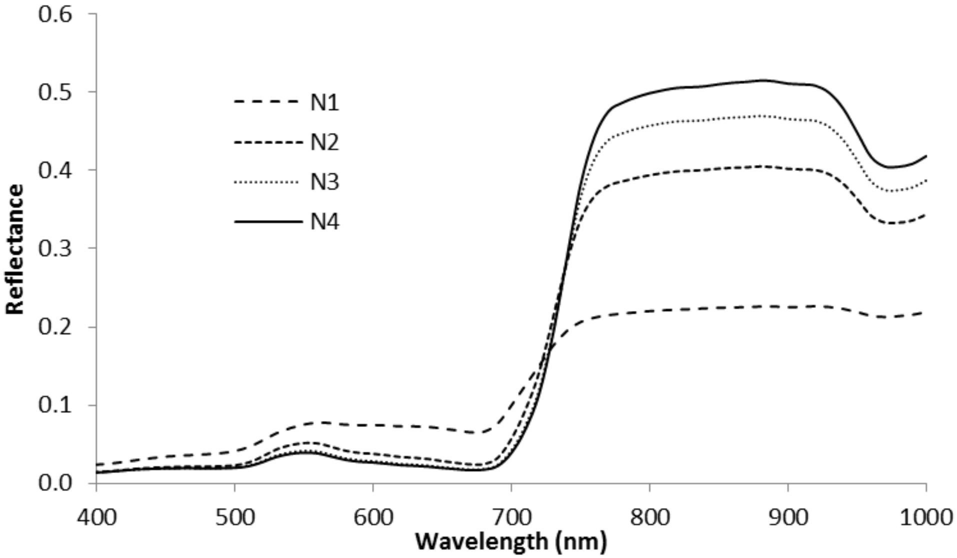
Height estimation from DEM/DSM

- Measurements taken May 22nd
- Only central 2m x 8m of each plot analysed
- Correlation very good when crop is >60cm
- Short/thin plots not so good



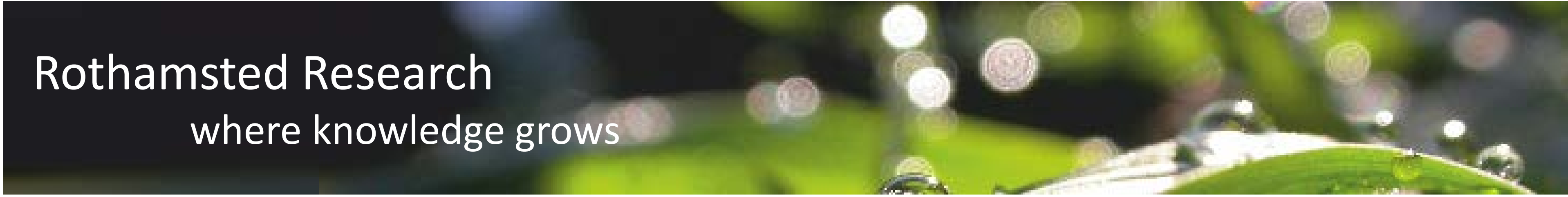
Data collection 2015

- Spectral reflectance weekly
- Date of anthesis
- Senescence
- Canopy height
- N & mineral uptake during GFP
- Aerial images
- Final harvest grain and straw yield



Thanks

- WGIN team
- Rothamsted Farm staff
- Saroj Parmar, March Castle, Grzegorz Kulczycki, Adam Michalski



Rothamsted Research

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WGIN3 Management Meeting

17th JULY 2015

Screening germplasm for resilience to aphids (WP2.3)

Lesley Smart

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 aphid (Sept 15)

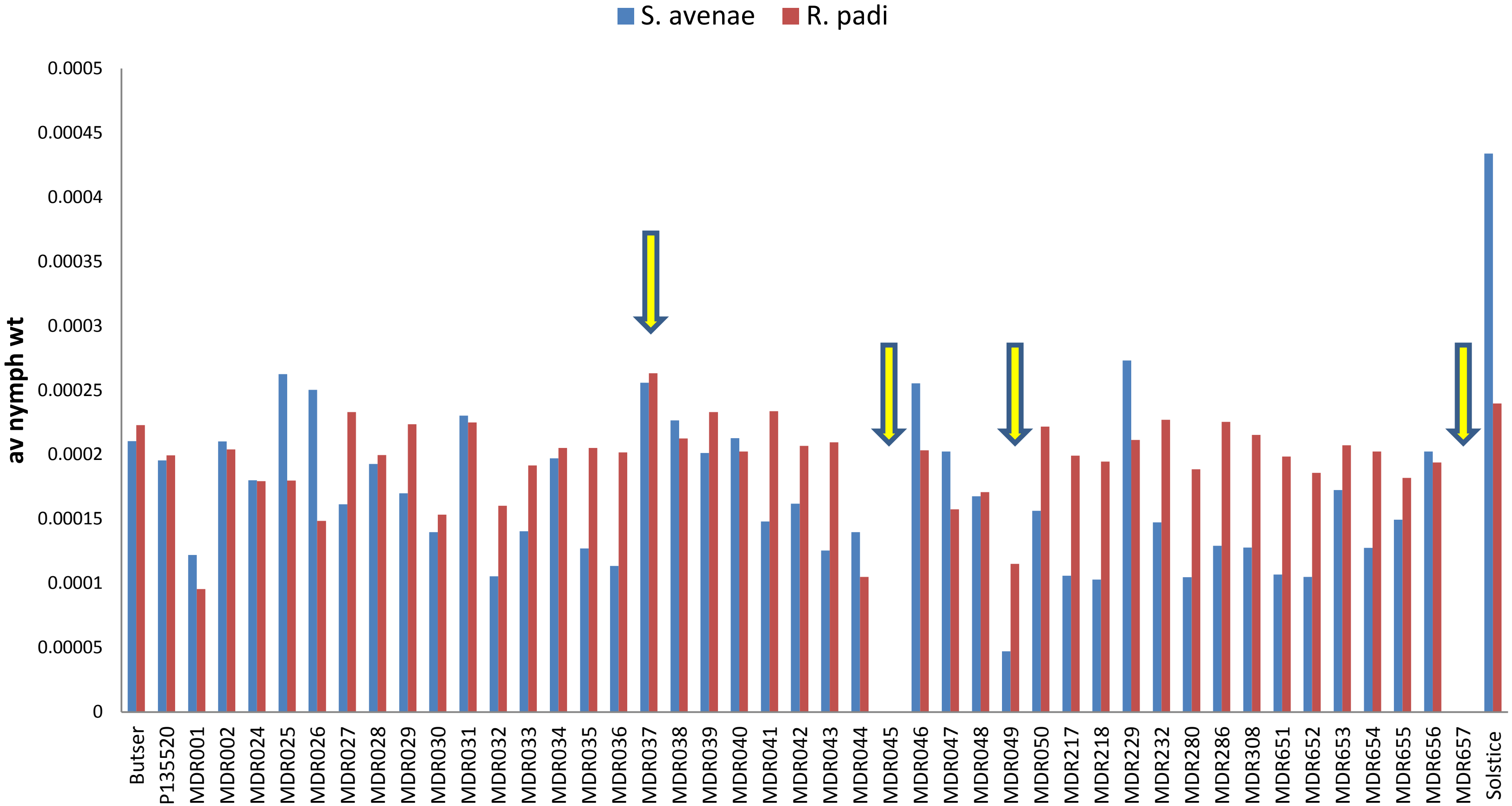
- Focus on *Triticum monococcum* lines as these provided the most promising leads for partial resistance to cereal aphids from previous work

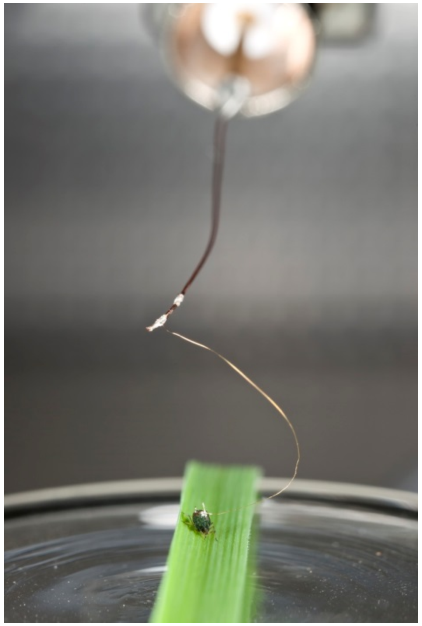
- Crosses made by Mike Hammond-Kosack: MDR037 x MDR045, MDR049 and MDR657

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

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

Nymph weight on *Triticum monococcum* lines

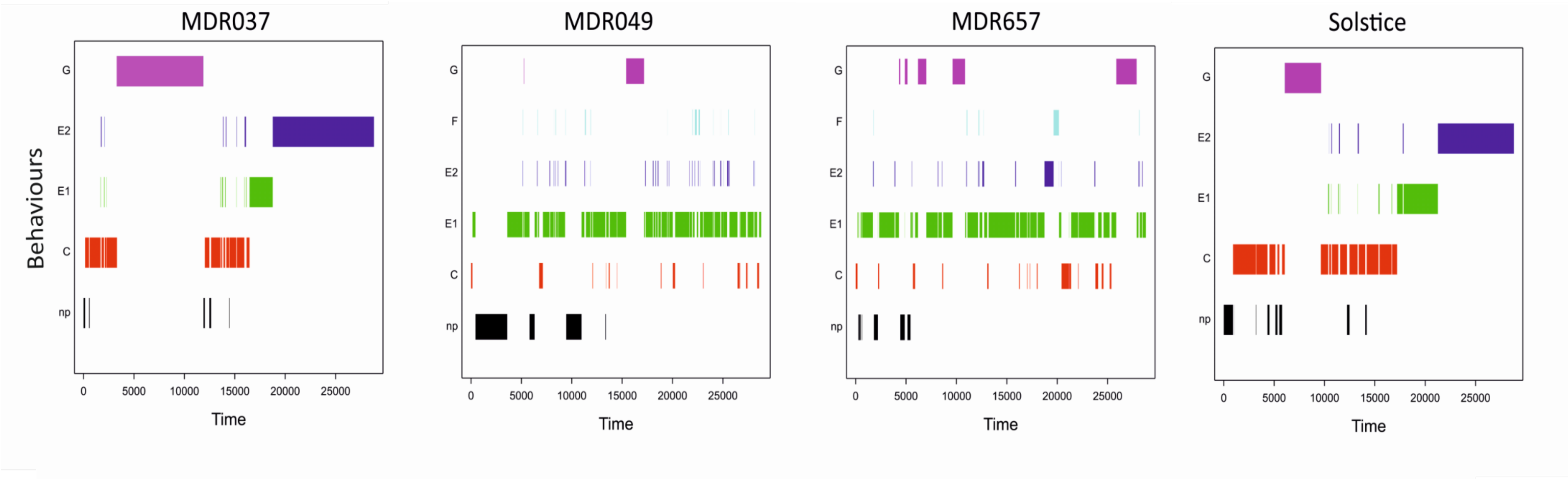




Eight hour EPGs for a representative replicate of *R. padi* on each of four wheat varieties (MDR=*Triticum monococcum*).

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

Work by Alex Greenslade

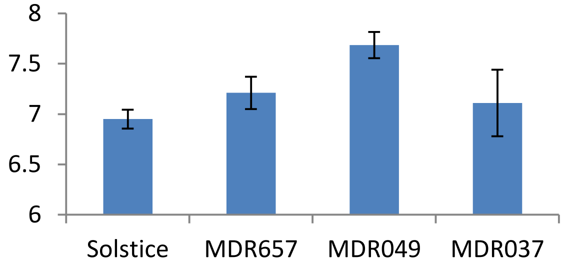


Fecundity assays – Intrinsic rate of increase (r_m)

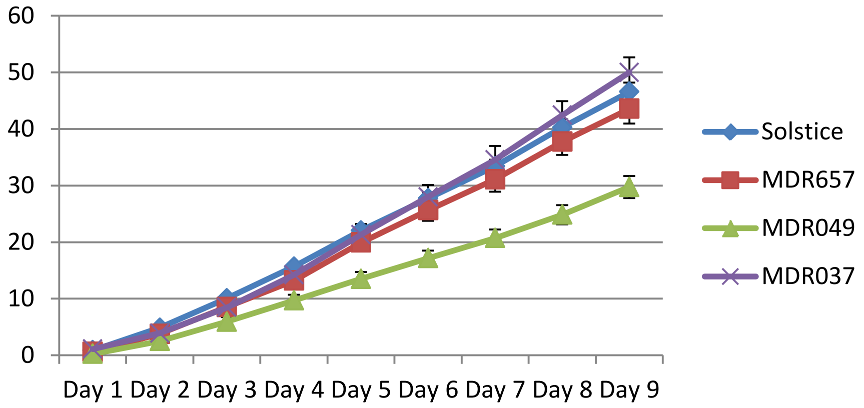
r_m = (ln(FD)/D) x C (0.74) (Wyatt and White, 1977)

Rhopalosiphum padi – no nymphs on MDR045

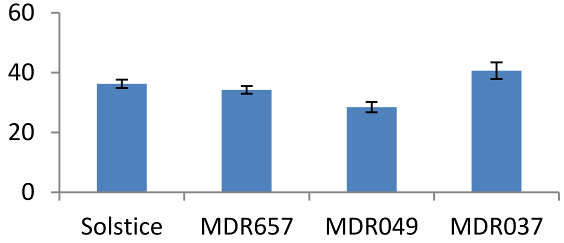
D (days from birth to 1st nymph production)



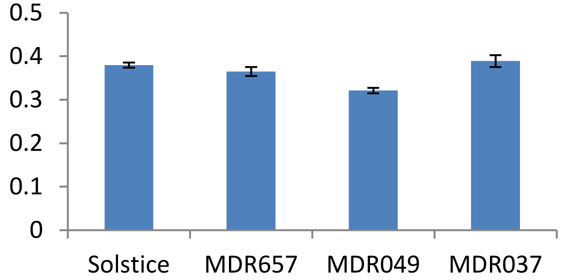
Cumulative nymph production



FD (nymphs produced over time D)



r_m



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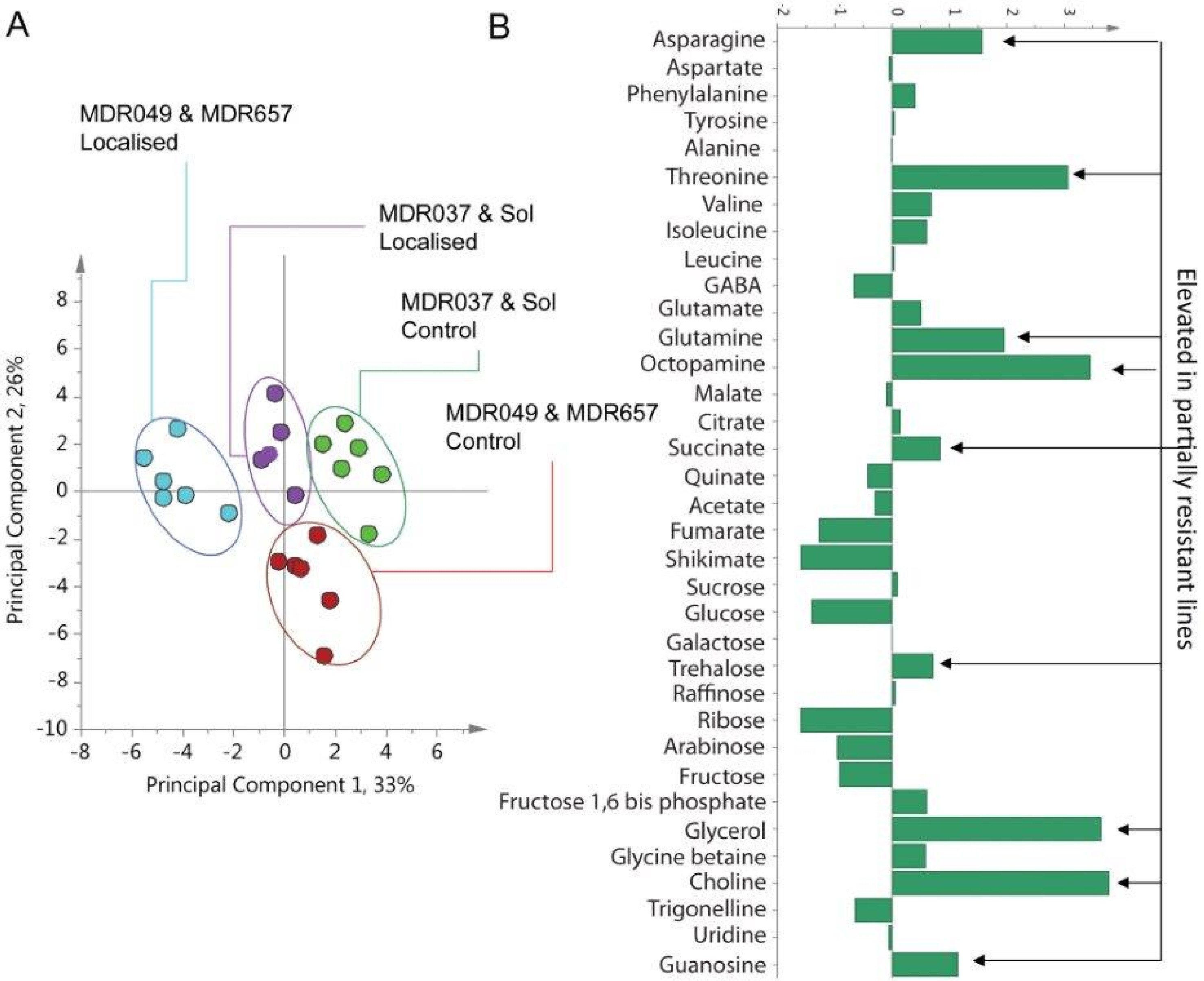
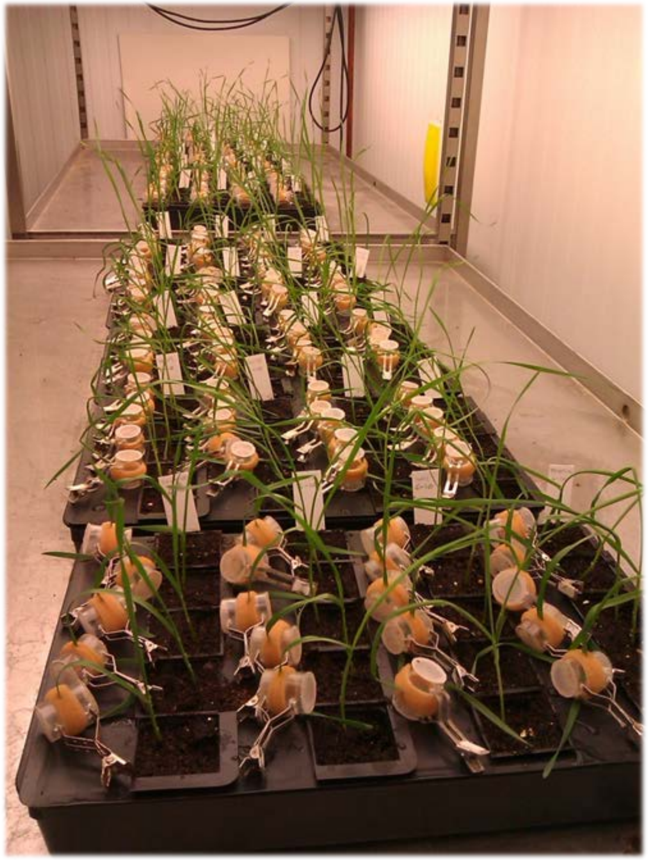
ROTHAMSTED RESEARCH

Metabolomic Analysis

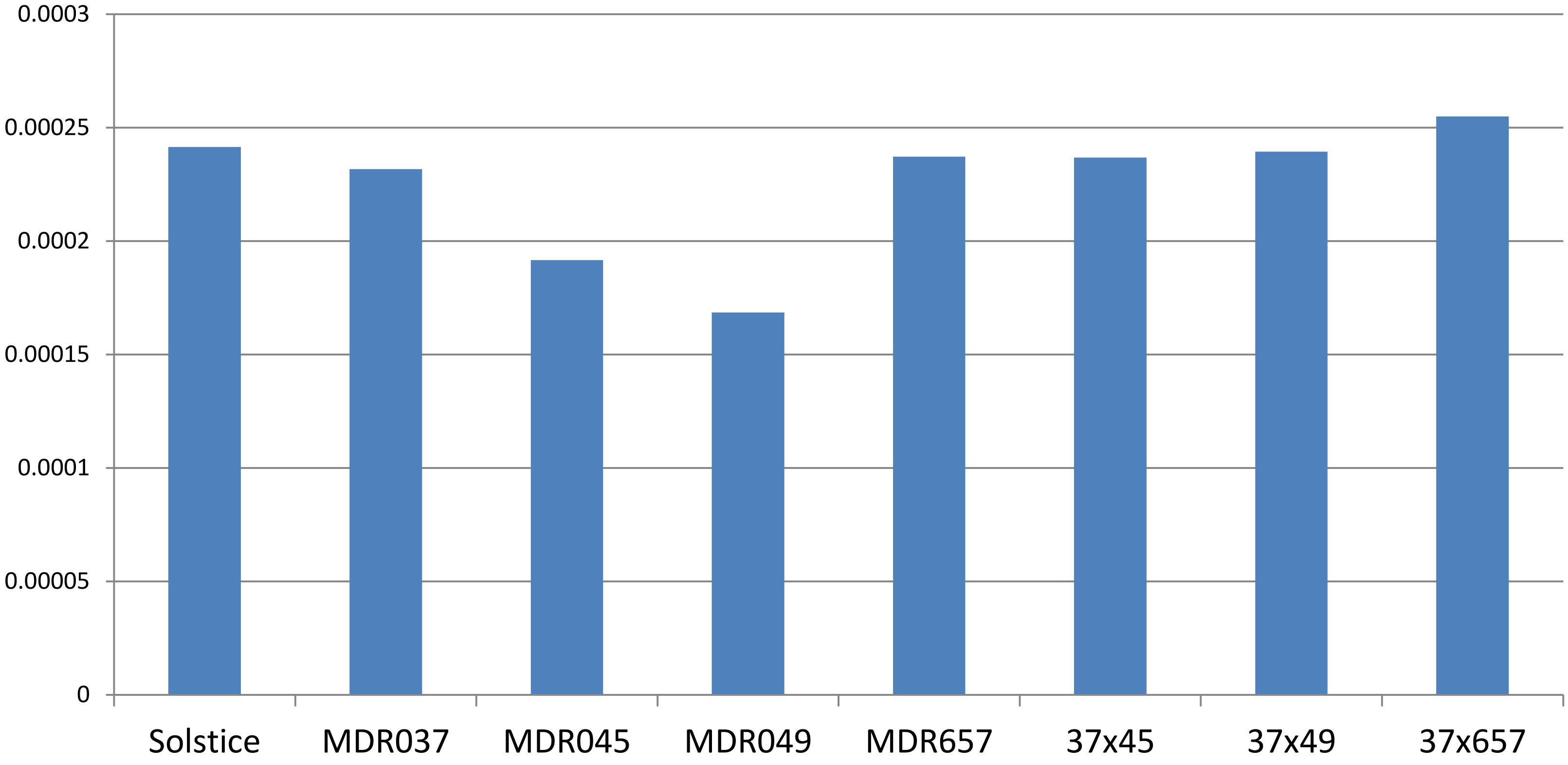


- F1 generations of crosses, MDR037 x MDR045, MDR049 and MDR657, tested in the phenotyping screen against both aphid species



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	

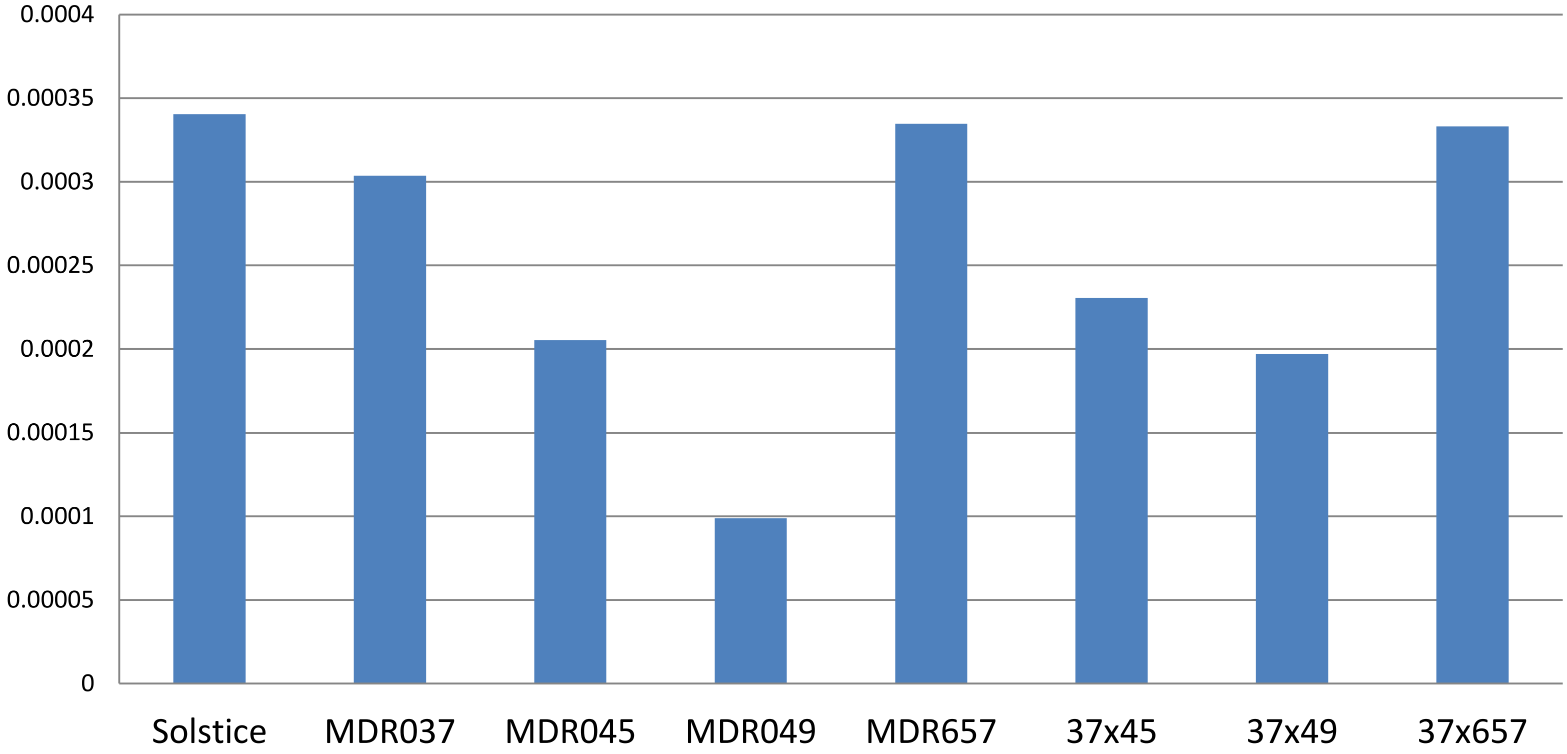
Rhopalosiphum padi mean nymph weight (mg) after 6 days on *T. monococcum* lines and crosses



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* lines and crosses



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

Summary

- Clear difference in feeding behaviour as well as distinct metabolic phenotypes for partially-resistant and susceptible plants (both before and after 24h aphid infestation)
- Further work planned to investigate effects of some chemicals against aphids in feeding bioassays.
- 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 just been harvested and will be tested in phenotyping screen and taken to further generations.

Acknowledgements

Gia Aradottir
and Mike Hammond-Kosack

Colleagues now moved to other projects

Alex Greenslade

Janet Martin

BBSRC bioscience for the future

ROTHAMSTED RESEARCH

WGIN 3
Resistance to take-all and foliar diseases

Vanessa McMillan

Kim Hammond-Kosack

WGIN MM 17th July 2015

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**

Yellow Rust

- Wheat yellow rust = *Puccinia striiformis* f.sp. *tritici*
- Obligate biotrophic pathogen
- Yield losses of up to 50%
- UK Cereal Pathogen Virulence Survey

Year	Variety
2000	Robigus
2008	Solstice
2011	KWS Sterling
2011	Warrior

Objective 3: Examine the resistance of *Triticum monococcum* to yellow rust

Background: *T. monococcum* grown at RRes since 2004, but never any obvious yellow rust infections

- Total *T. monococcum* collection to be assessed for yellow rust resistance under field trial conditions

- Collection to be genotyped by University of Bristol – association analysis approach

RRes *Triticum monococcum* collection

Total number	323 (Vavilov, USDA, IPK)
Country of origin	35
Spring habit	229
Winter habit	86

* Enough seed of 263 accessions for yellow rust field trial

Field trial design

- *T. monococcum* collection (263 accessions) sown in field trial 31st October 2014 (one replicate per accession)
- Spreader rows of the highly susceptible hexaploid cultivar Robigus sown in between *T. monococcum* plots

Tm Robigus Tm

25th March 2015

natural yellow rust infection

- 18% plots did not establish successfully
- A total of 216 accessions could be scored for foliar disease

Yellow rust inoculation

- Three yellow rust isolates obtained from NIAB

  Solstice isolate 08/21
  KWS Sterling isolate 11/140
  Warrior isolate 11/08

- Grow Robigus seedlings for 2 weeks (until GS 12)

- Inoculate with yellow rust spore:talc mixture (1:19)

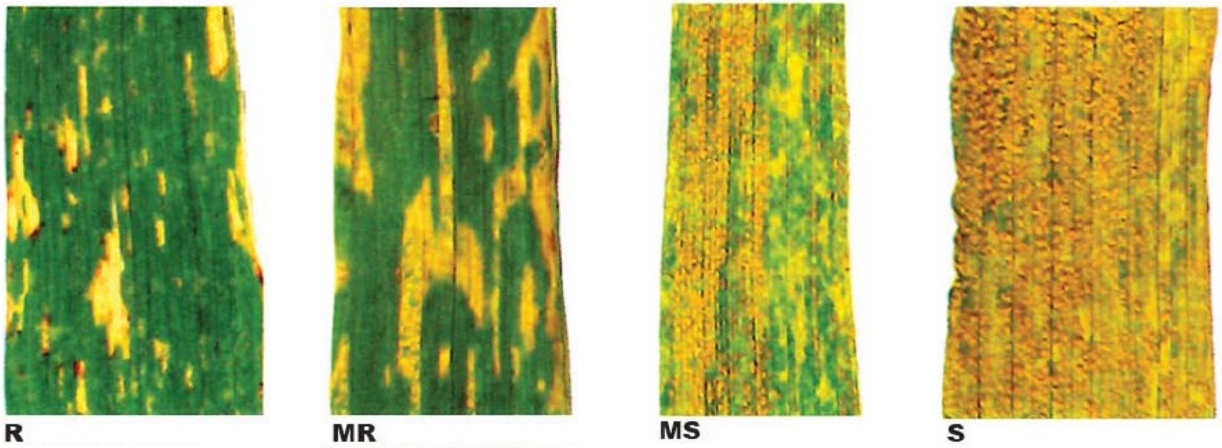
- Cover trays with plastic bag (to keep high humidity) and cold treatment for 48 hr

- Grow at room temp for 2 weeks until symptom development and then hand planted into Robigus spreader rows in field trial 25th March 2015

Yellow rust disease assessments

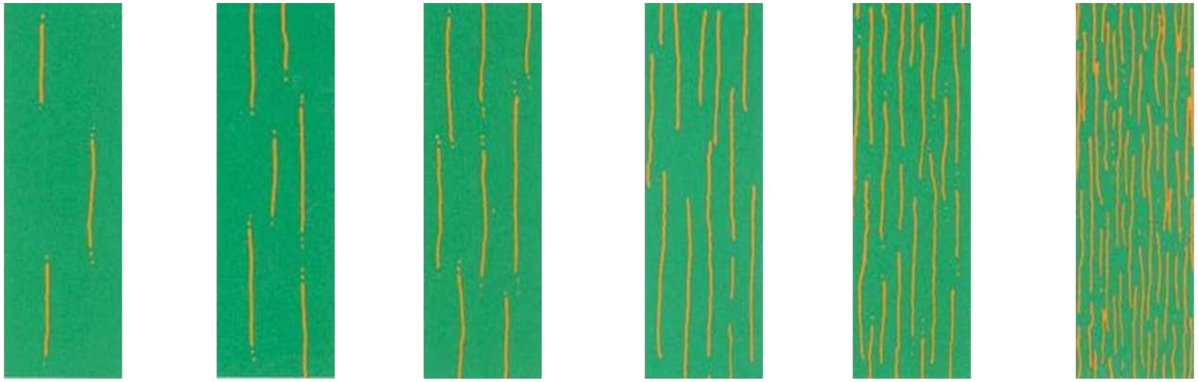
Field response

0 = no infection



Disease severity

Modified Cobb scale
(percentage of rust
infection on plant or leaf)



severity 5% severity 10% severity 20% severity 40% severity 60% severity 100%

http://wheatdoctor.org/scoring-stripe-rust

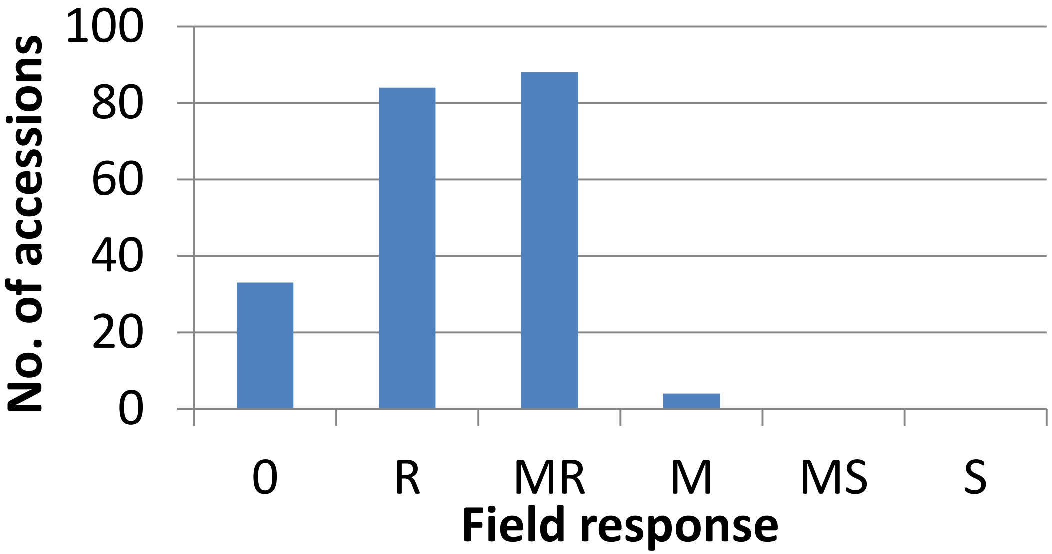
Yellow rust disease assessments

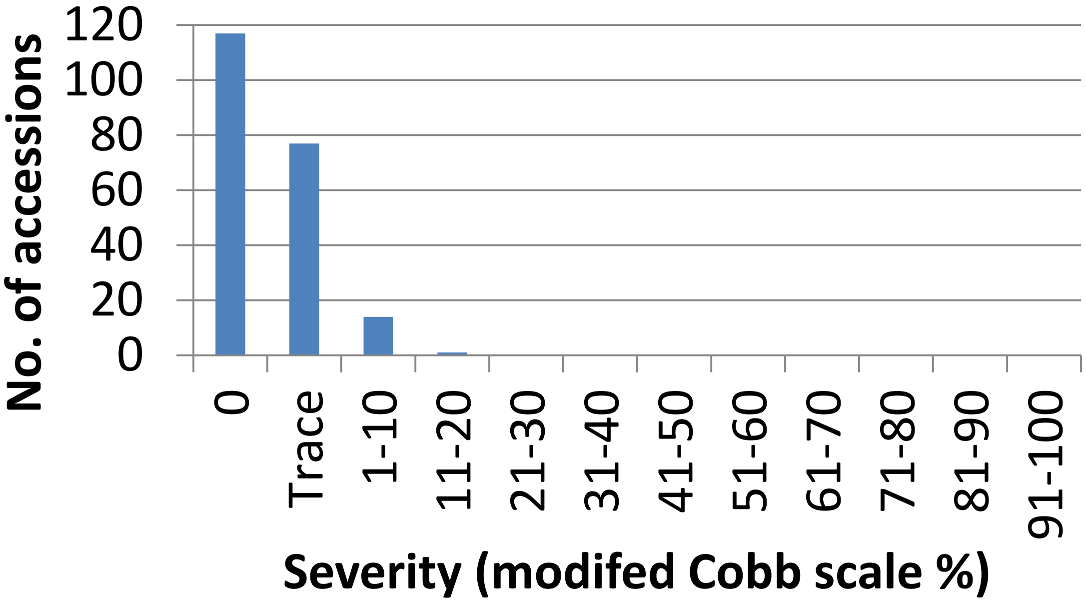
27th April 2015 – tillering GS 26-29

27th May 2015 – flag leaf emergence GS 39-40

26th June 2015 – mid/end of flowering GS 65-69

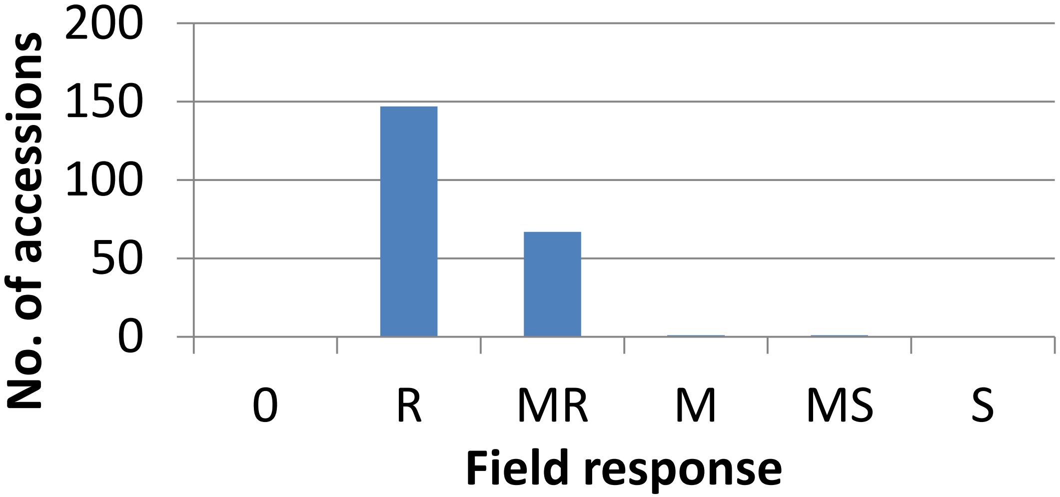
27th April 2015 – tillering GS 26-29

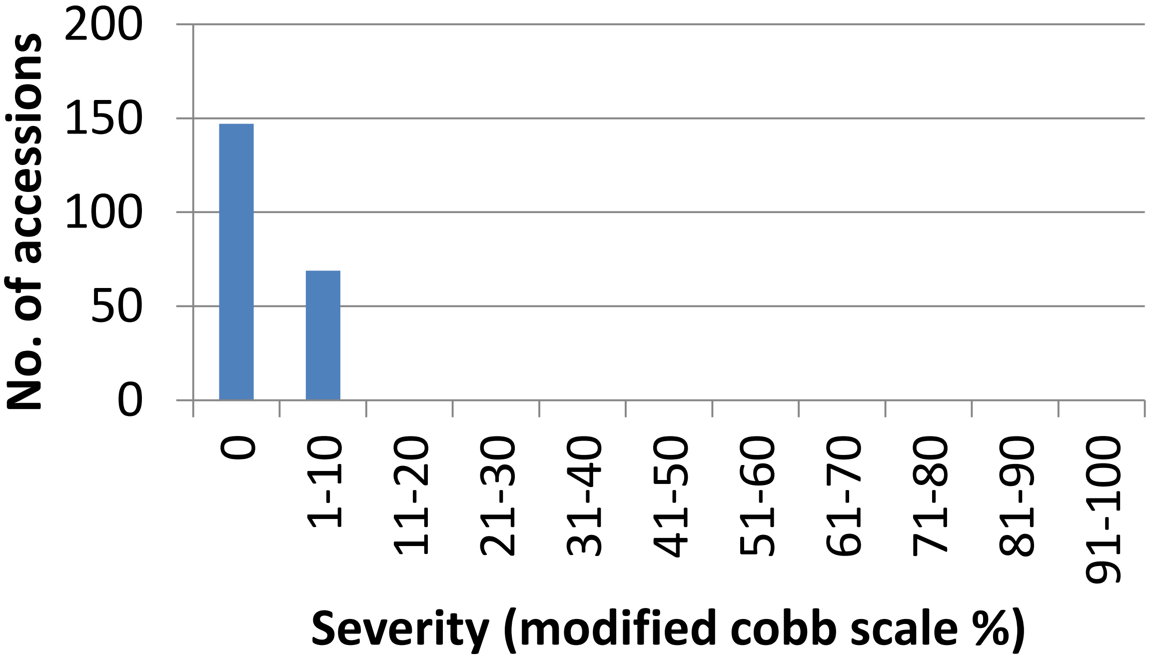




- Robigus = 30% disease severity
- No highly susceptible *T. monococcum* but sporulation visible on ~ 40% of accessions

27th May 2015 – flag leaf emergence GS 39-40





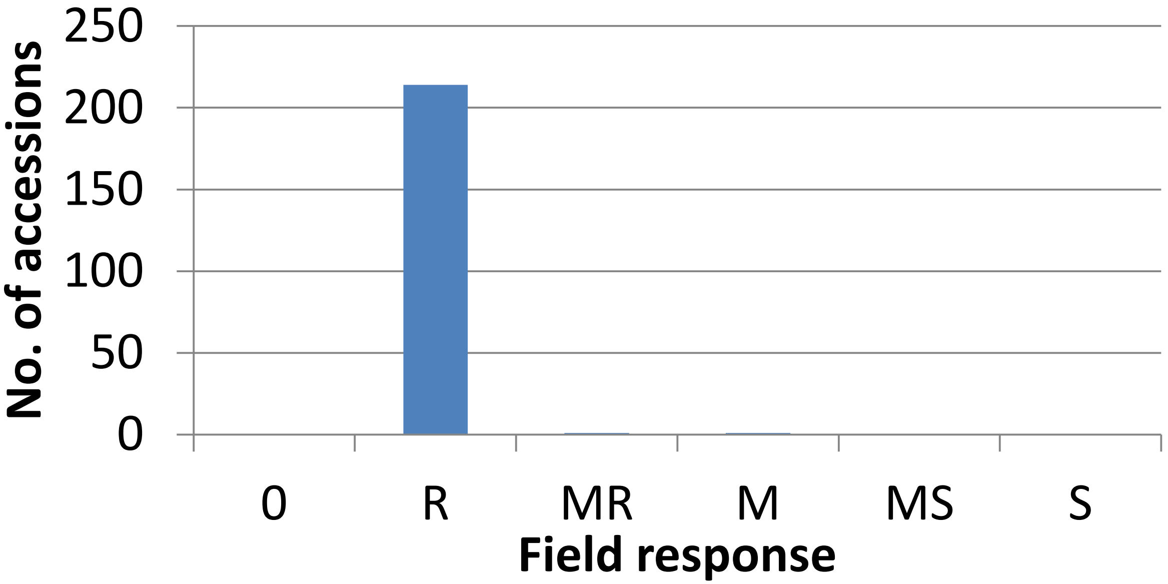
- 2nd leaf disease assessments

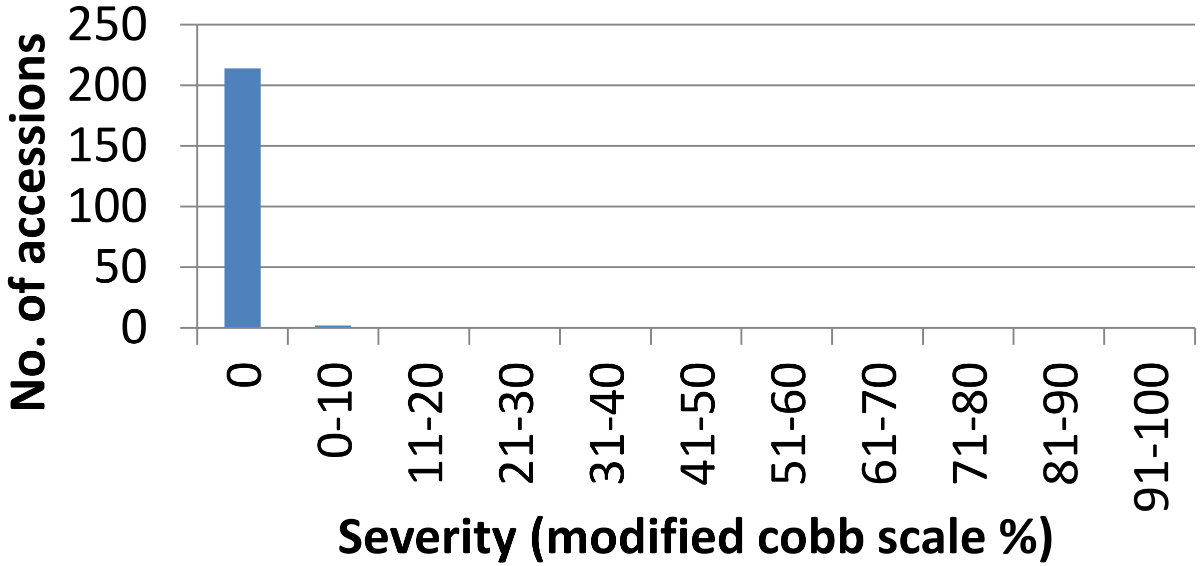
- **Robigus**

Flag leaf = 0-5%
2nd leaf = 30-100%

- Most accessions had a resistant phenotype or low levels of rust on 2nd leaf (1%)

26th June 2015 – mid/end of flowering GS 65-69





- Flag leaf disease assessments

- **Robigus**
  Flag leaf = 60-100%

- 99% accessions had a resistant phenotype with some chlorosis and necrosis visible on flag leaf

- Two accessions showed yellow rust sporulation on flag leaf

Yellow rust resistance - summary

- Diverse *T. monococcum* accessions all **highly resistant** to yellow rust
- Low levels of yellow rust (1% severity) detected at tillering and stem elongation/flag leaf emergence for many accessions
- 99% of accessions showed resistant phenotype at flowering (some chlorosis/necrosis, no yellow rust sporulation)
- Two accessions showed yellow rust sporulation on flag leaf at flowering

MDR634: 10% - probably not *T. monococcum,* mistake in seed store
MDR288: 2% - also showed stem purpling and powdery mildew infection
Country of origin = Turkey

Possible Next Steps – to discuss

- Infected leaves from MDR288 put into -80°C freezer for future sequencing of the yellow rust genome

- Trial to be hand harvested and repeat sown for 2015/2016 field season

- Mapping populations created between MDR288 (S) and resistant accessions to map resistance / susceptibility loci

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

Background

- WGIN 2: 3rd wheat, Take-all field experiment in 2008 – Watkins collection (740 lines) – Richard Gutteridge
- Single replicate of each Watkins line
- No fungicides
- Trial assessed for yellow rust, brown rust, septoria and powdery mildew infection and plant samples taken for take-all assessments on the root systems

Watkins 2008 field trial

High foliar disease pressure – brown rust, powdery mildew, yellow rust and Septoria

Watkins 2008 field trial

Background

- 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

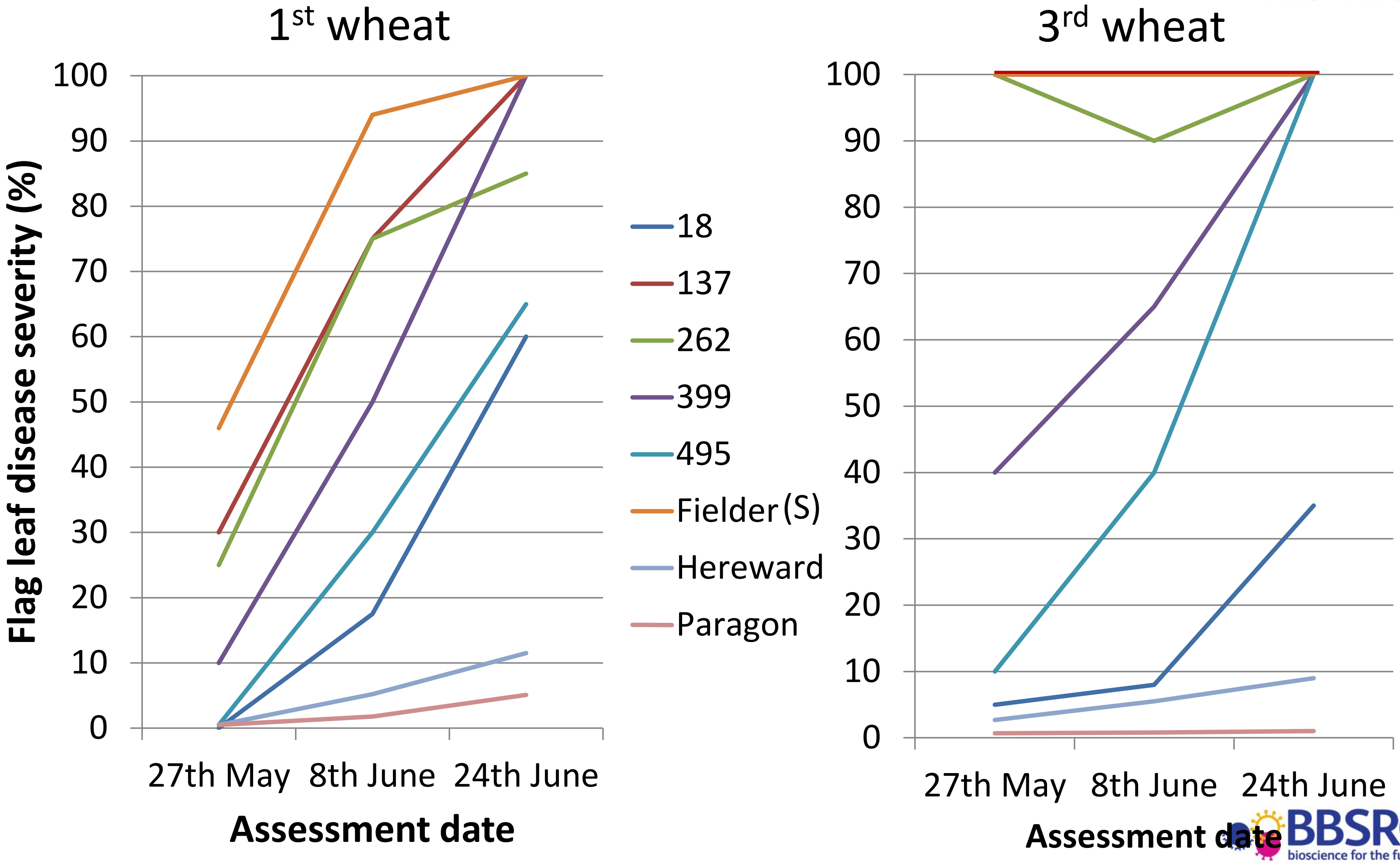
Watkins foliar disease field trial 2015

11th May 2015   3rd wheat Bylands

Yellow rust dominant disease that developed across 2015 field trials

5/10 Watkins lines very susceptible to yellow rust

ROTHAMSTED
RESEARCH



1st wheat

3rd wheat

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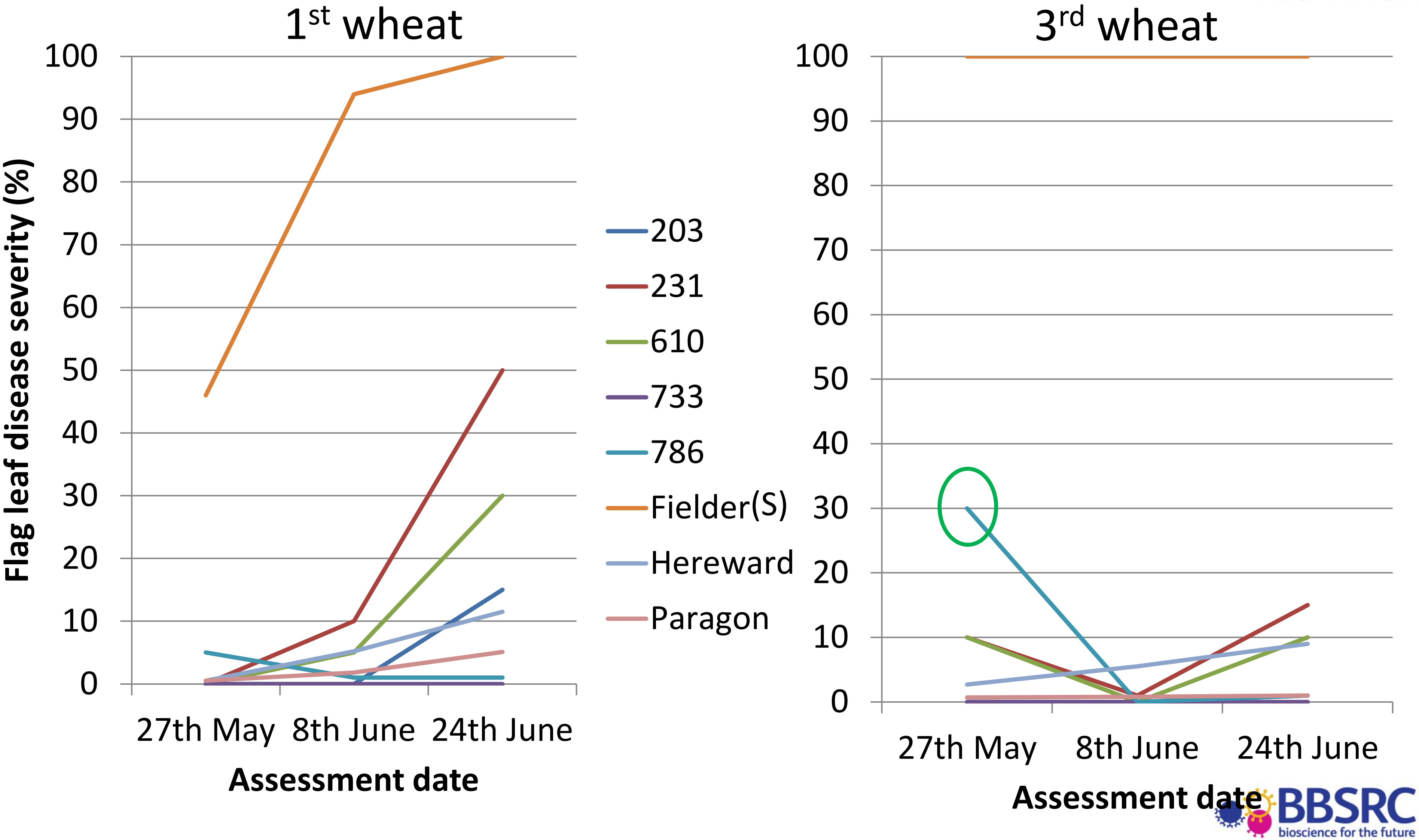
5/10 Watkins lines show some resistance to yellow rust



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)

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	MR	8	31
231	M/MR	8	54
495*	MS	6	13
610	M/MR	6	35
733	0	6	46
Totals		**41**	**249**

* Included in crossing as low disease severity in May

Crossing carried out by Mike Hammond-Kosack

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 in the autumn and compared to foliar disease – evidence for an induced resistance response or not?

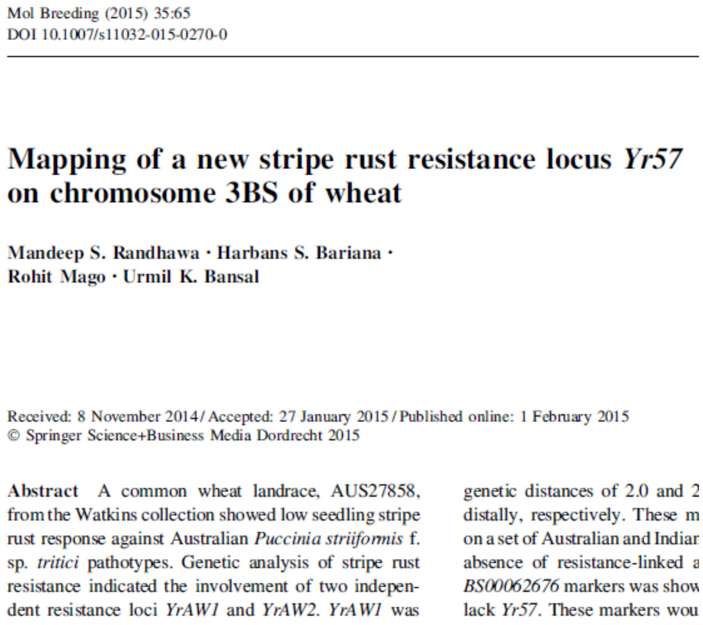
Possible Next Steps – to discuss

- Trial to be hand harvested and repeat sown for 2015/2016 field season

- Watkins 786 to be crossed with cv. Fielder in glasshouse or field 2016 (not included in 2015 field crossing due to high disease in May)

- Mapping populations to be progressed to F_2 and then screened for yellow rust resistance

- University of Sydney – evaluated Watkins wheat lines against Australian yellow rust isolates, need to identify which Watkins lines they have been working on



Mol Breeding (2015) 35:65
DOI 10.1007/s11032-015-0270-0

Mapping of a new stripe rust resistance locus *Yr57* on chromosome 3BS of wheat

Mandeep S. Randhawa · Harbans S. Bariana ·
Rohit Mago · Urmil K. Bansal

Received: 8 November 2014 / Accepted: 27 January 2015 / Published online: 1 February 2015

Abstract A common wheat landrace, AUS27858, from the Watkins collection showed low seedling stripe rust response against Australian *Puccinia striiformis* f. sp. *tritici* pathotypes. Genetic analysis of stripe rust resistance indicated the involvement of two independent resistance loci *YrAW1* and *YrAW2*. *YrAW1* was

genetic distances of 2.0 and 2
distally, respectively. These m
on a set of Australian and India
absence of resistance-linked a
BS00062676 markers was show
lack *Yr57*. These markers wou

Many thanks to

ROTHAMSTED
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Simon Orford (JIC)

defra
Department for Environment
Food and Rural Affairs

Wheat
Genetic
Improvement
Network

BBSRC
bioscience for the future

Take-all disease

Major root disease of wheat

Ascomycete soil-borne fungal pathogen
Gaeumannomyces graminis var. *tritici (Ggt)*

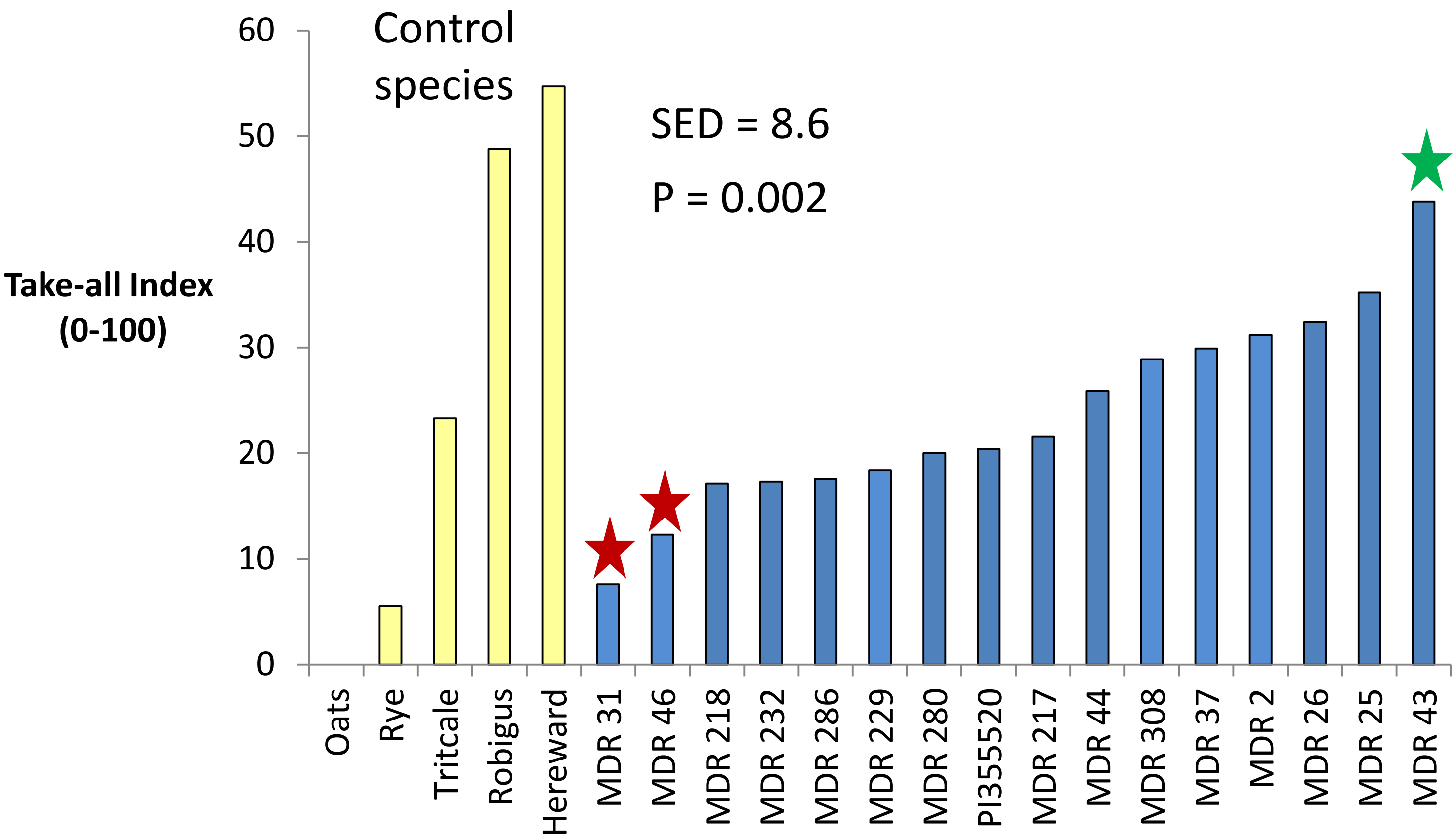
Take-all infected wheat seedling

Take-all patch showing stunting and premature ripening of the crop

Limited control options

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_4, taking forward to F_6 ⬅

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

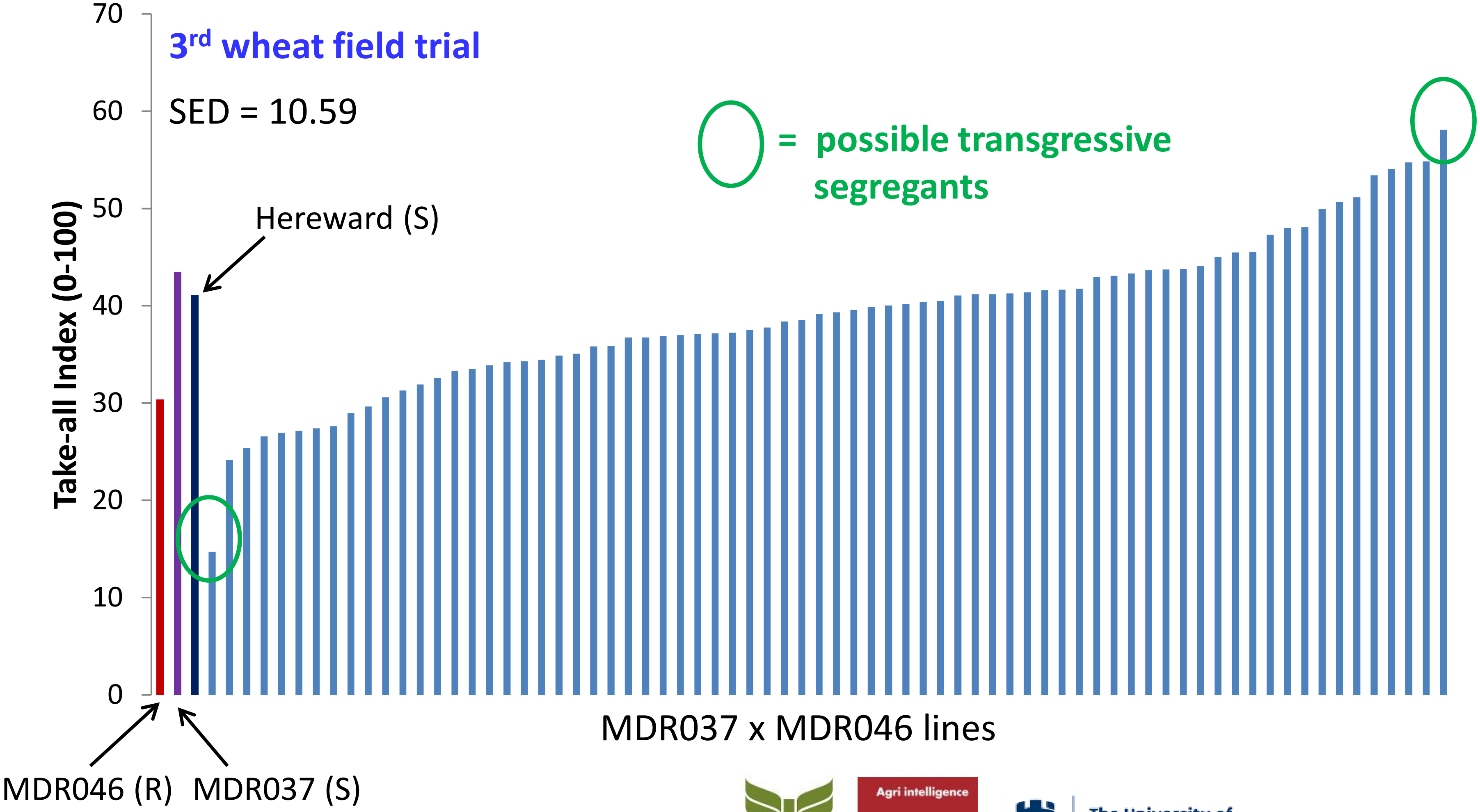
2013/2014 field trial (3rd wheat situation):

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

- Plant samples taken at GS 75

PhD student Sarah-Jane Osborne

MDR037 (S) x MDR046 (R) mapping population



3rd wheat field trial

SED = 10.59

= possible transgressive segregants

PhD student Sarah-Jane Osborne

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Exome Capture

Kim Hammond-Kosack

MYcroarray

Ann Harbor, Michigan, USA

17th July 2015

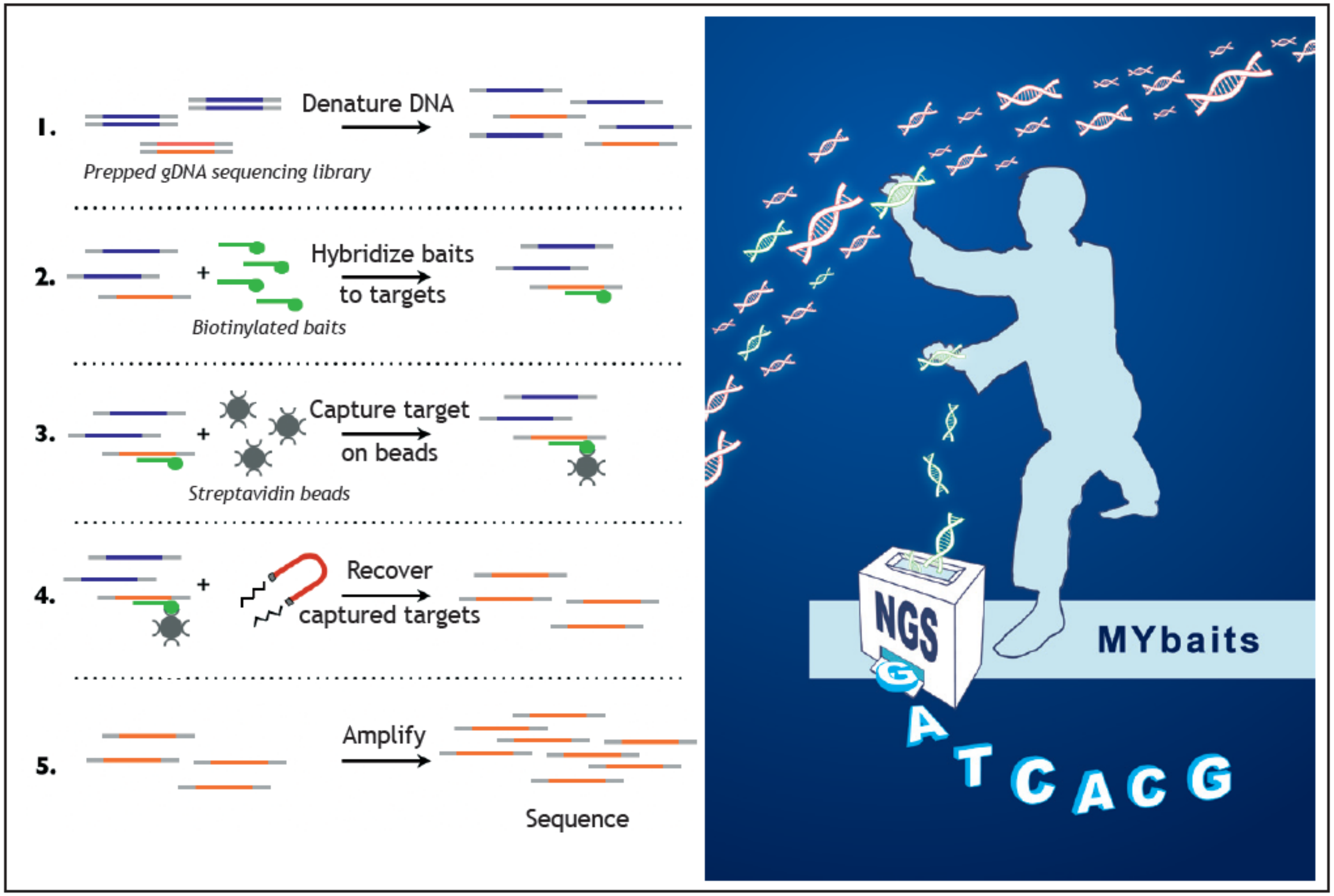
Exome Capture

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.**

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	Specific traits
Avalon	**Garcia**
Cadenza	**Watkins 777**
Paragon	
Chinese Spring	
Kronos (tetraploid)	
Diploids	

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

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

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

Who to be involved ?

So far

JIC – Simon Griffiths, Cristobal Uauy*

NIAB – Alison Bentley

RRes - Kim Hammond-Kosack, Andy Phillips*

* BBSRC BBR wheat tilling project

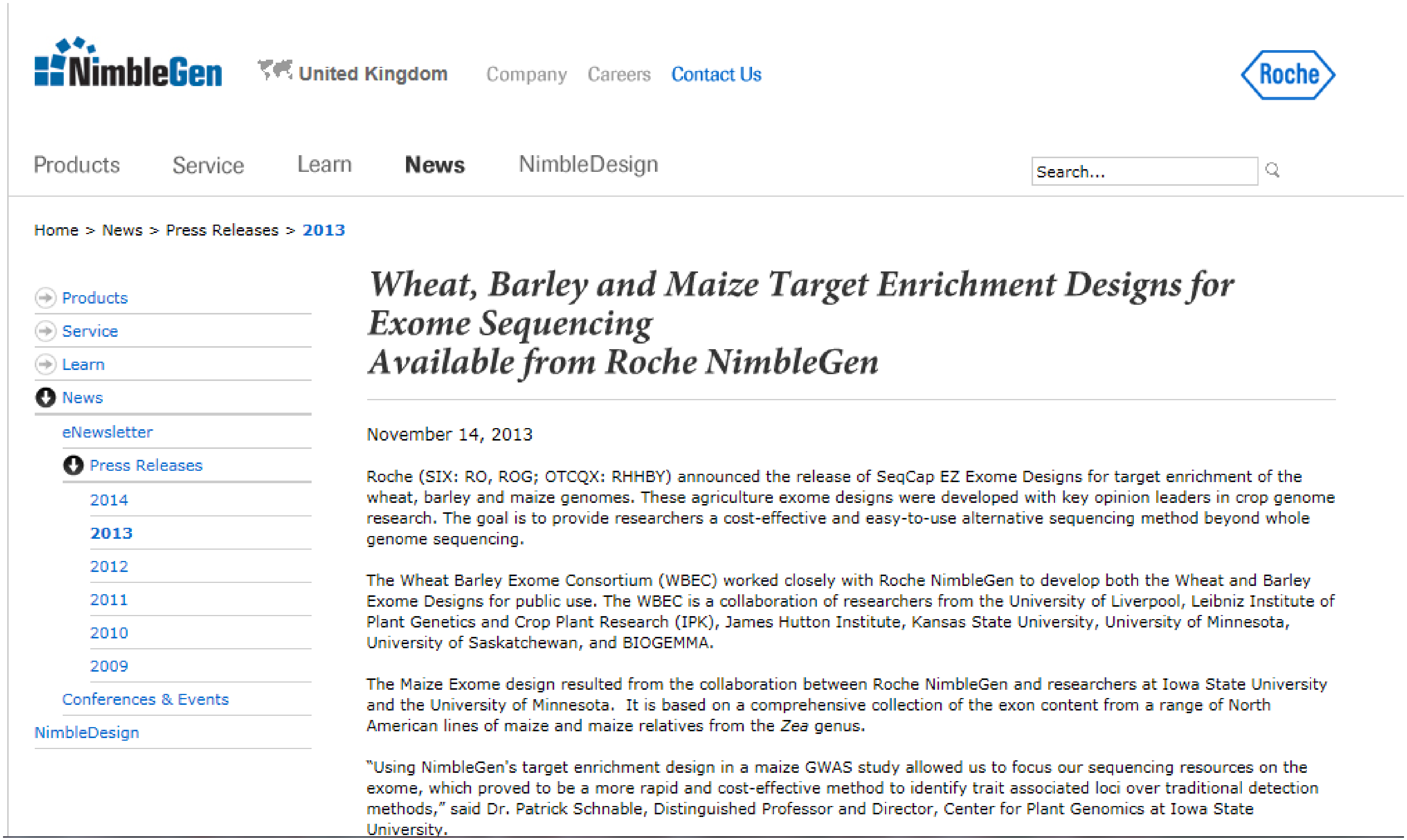
Exome capture – next steps

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).**