Wheat
Genetic
Improvement
Network

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Tools, resources, genotyping and phenotyping

Clare Lister and Simon Griffiths

12/1/2017

Simon Orford, Alba Farre-Martinez, Ji Zhou

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WGIN3 Projects: Griffiths' Lab

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

WGIN3 Projects: Paragon Library

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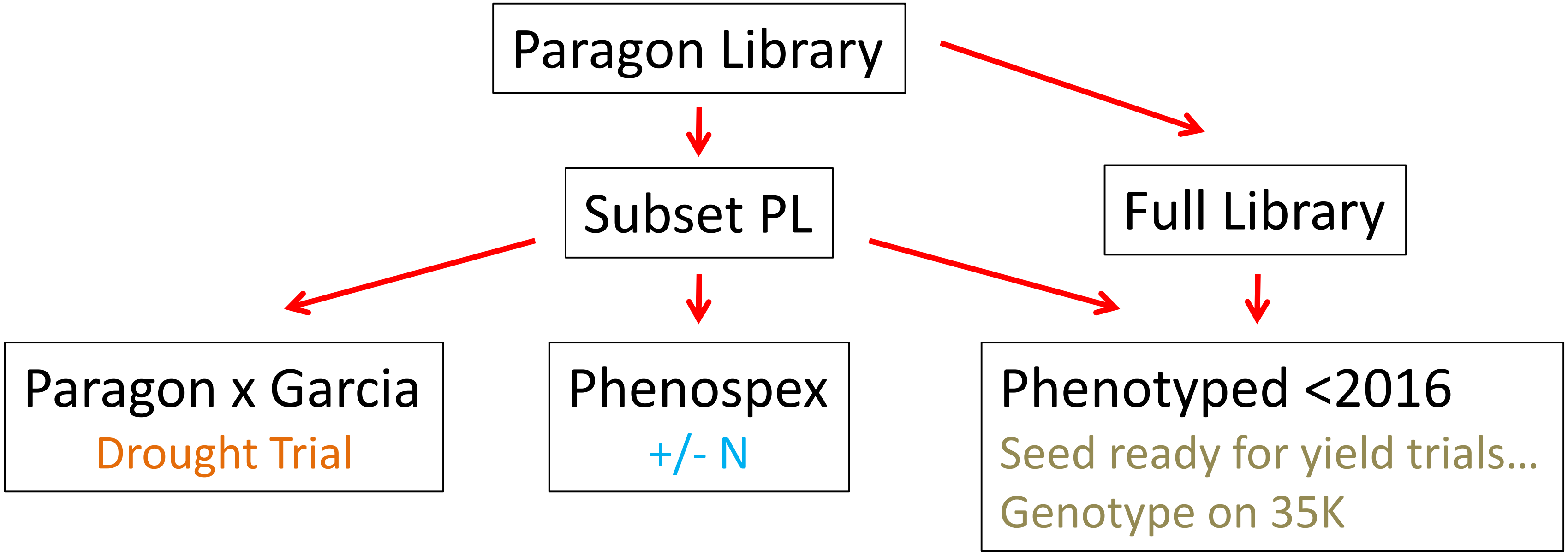
WGIN3 Projects: Paragon Library

NILs in the genetic background of Paragon:
Rht-D1, Rht-B1, Rht8, Ppd-B1, Ppd-D1, Lr19, 1BL.1RS, 8 eps QTL, *Vrn1, Vrn3*, 3N, grain shape QTL, QTL from the Watkins collection (BBSRC WISP), selected WGIN mutants.



Paragon Library

Subset PL

Full Library

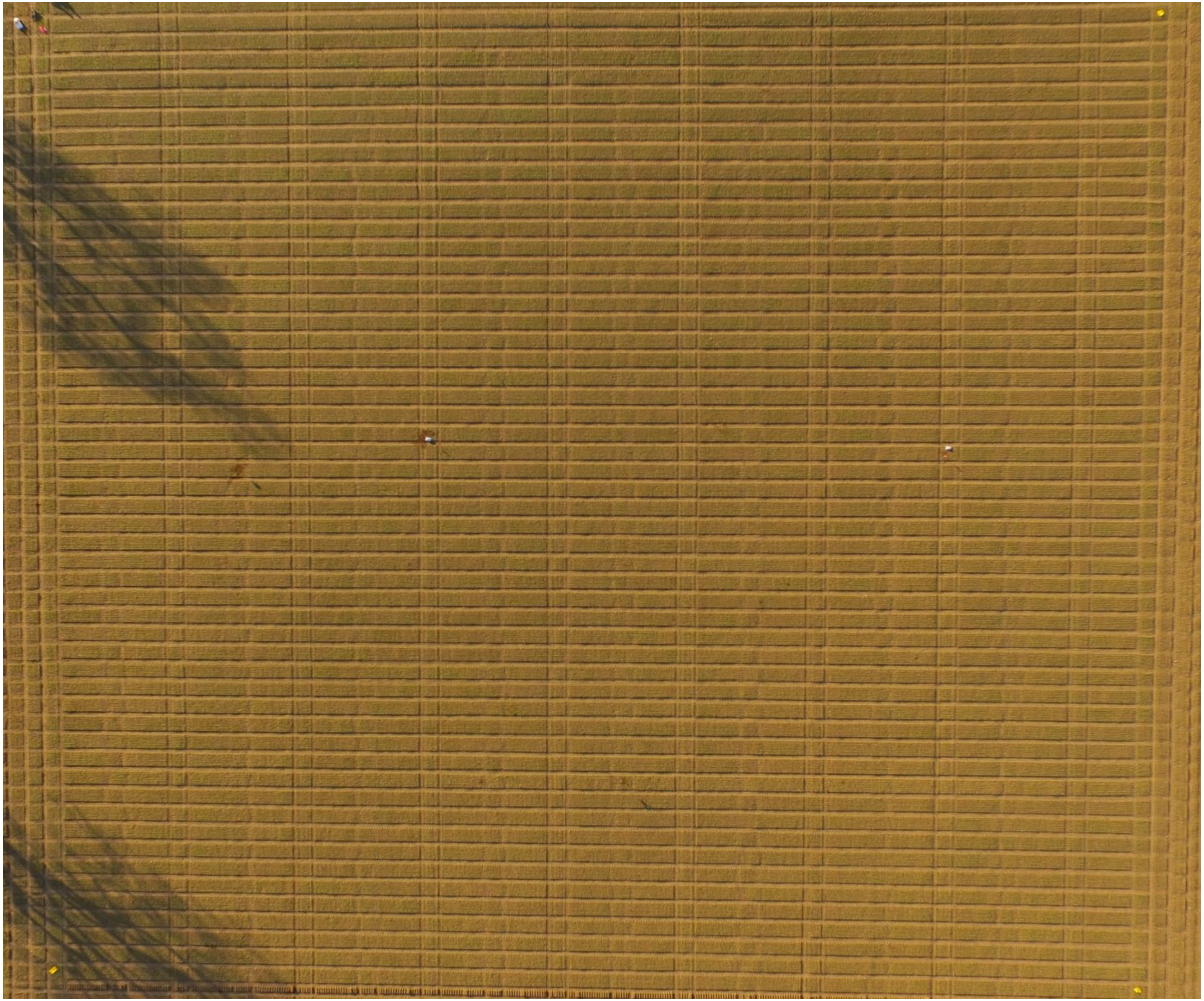
Paragon x Garcia
Drought Trial

Phenospex
+/- N

Phenotyped <2016
Seed ready for yield trials…
Genotype on 35K

1. Dissecting UK drought tolerance in Paragon x Garcia

- Drought Trial repeated 2016-2017
- Riverside Field, Church Farm, Bawburgh - field with lighter, sandier soil and gentler slope
- Monitoring soil water content
- Will monitor regularly with UAV (and Rothamsted drone!)
- Possibility of spring sowing?



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

Subset of Paragon Library

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

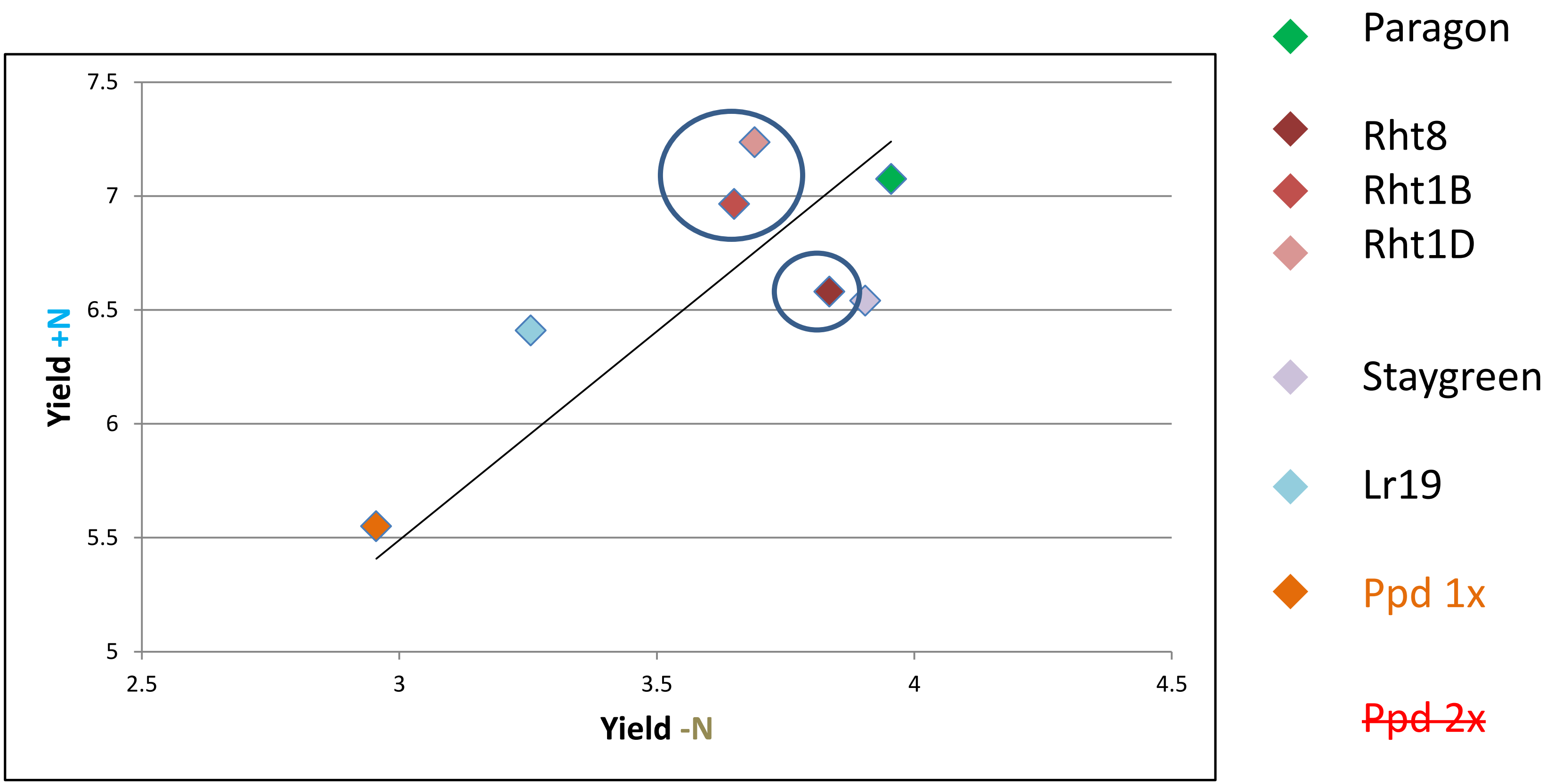
*Leaf rust resistance gene on 7DL, derived from *Agropyron elongatum*

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

- Subset PL lines drilled under Phenospex (2015-2016)
  - Two randomised blocks of 16
  - One treatment – all plots + nitrogen (40 kg/h)
  - Two subsequent treatments +/- additional nitrogen (250 kg/h)

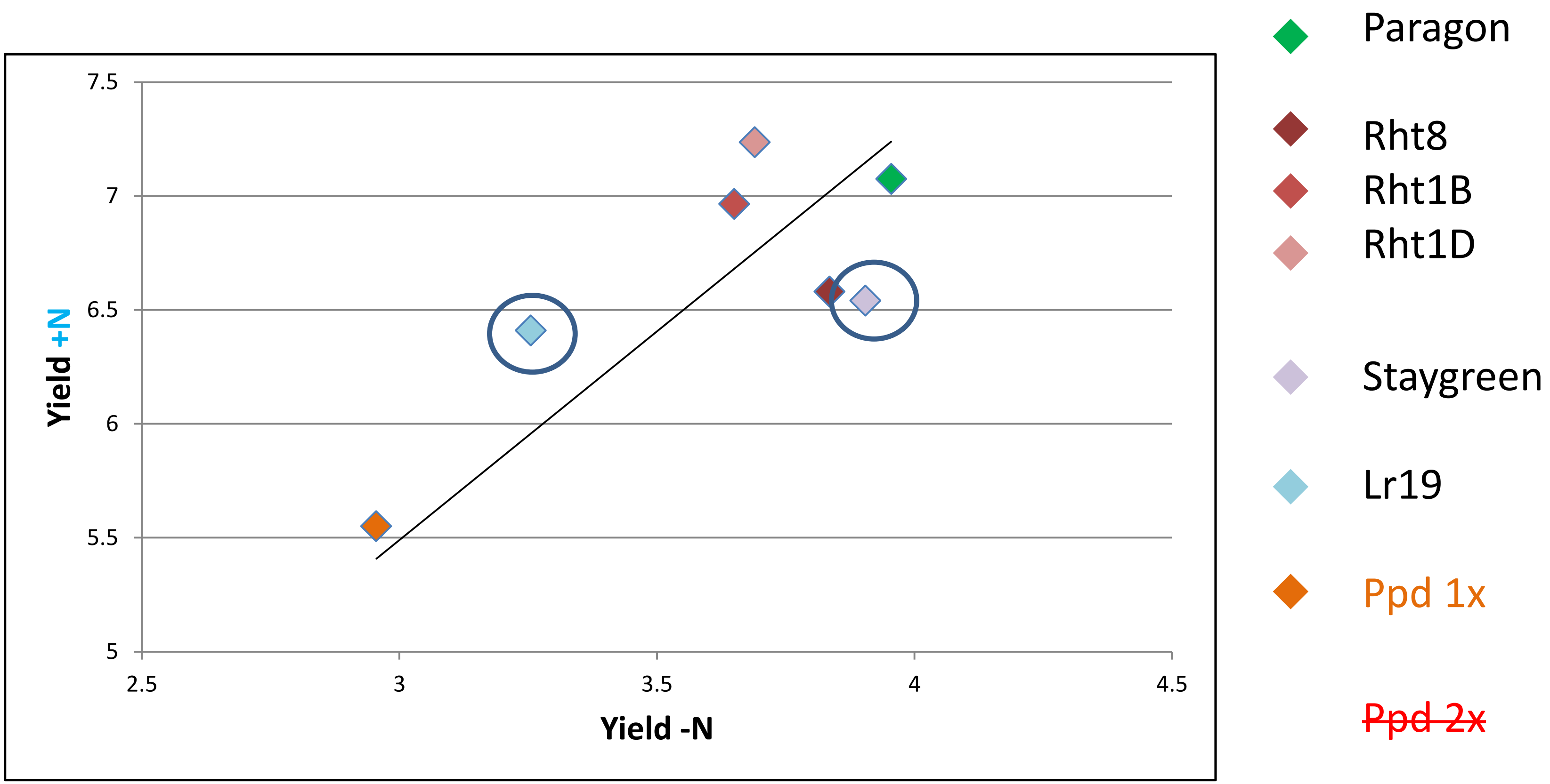
Paragon
Lr19 Kamb1
Staygreen
Ppd 1x Early
Ppd 2x Early
Rht 8 Mara
Rht B1 Robigus
Rht D1 Alchemy

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



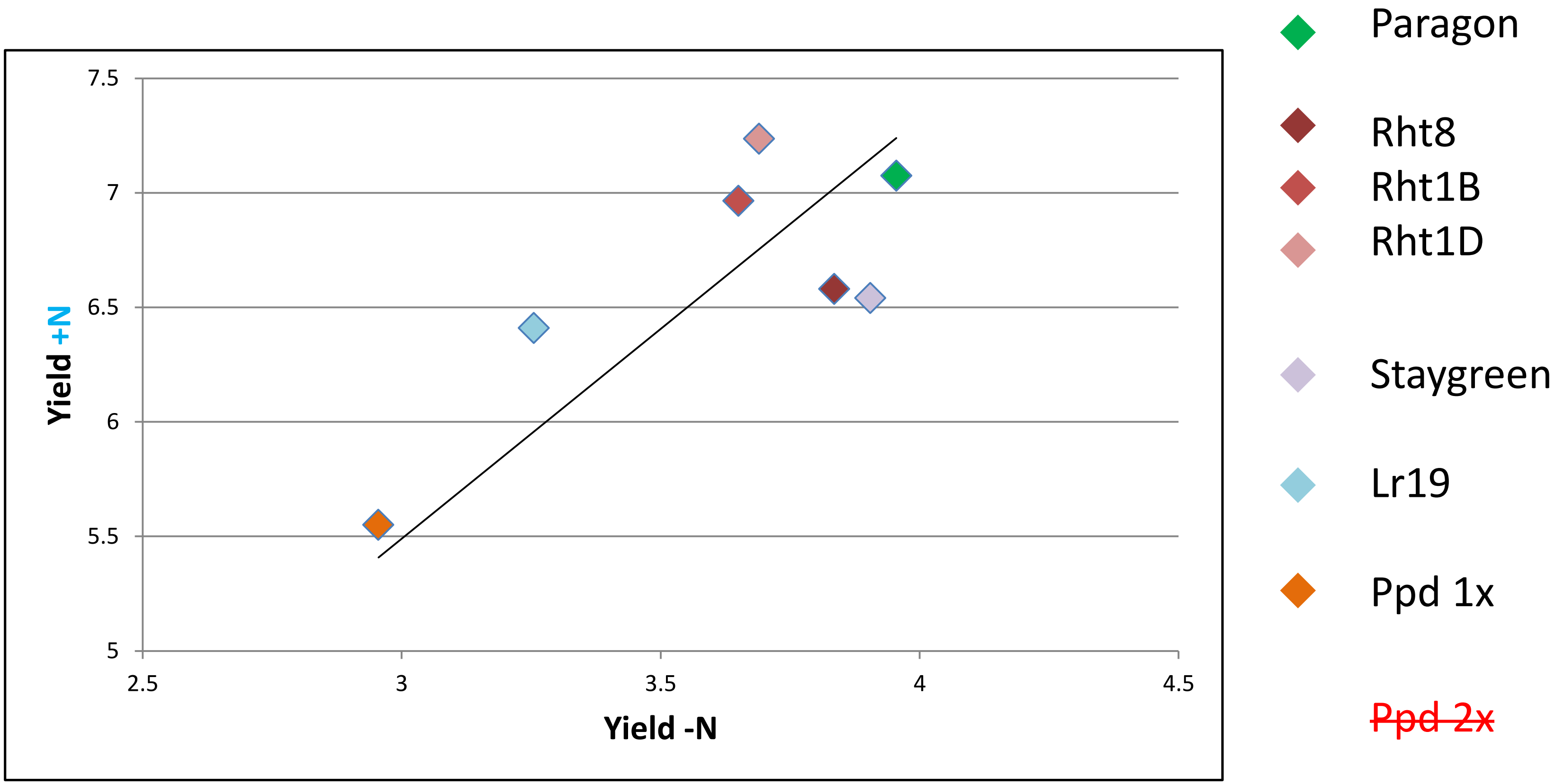
- RhtB1 and RhtD1 give low yield –N but high yield +N
- Rht8 gives high yield –N
- RhtB1/RhtD1 combined with Rht8 could produce more stability

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



- Staygreen mutant gives high yield **–N**
- Lr19 introgression shows a yield penalty **–N**
- Ppd 1x results unreliable due to lost seed

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



- JIC Phenospex Trial 2016-2017
- Drilled on land previously +/- clover
- Repeating +/- Nitrogen (not under Phenospex)

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

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Combining gibberellic acid-sensitive and insensitive dwarfing genes in breeding of higher-yielding, sesqui-dwarf wheats

G.J. Rebetzke*, D.G. Bonnett[1], M.H. Ellis[1]

- Reductions in plant height are associated with increase harvest index and wheat yields.

- Largest increase due to development of semi-dwarf wheats through introgression of the gibberellic acid (GA)-insensitive, Rht-B1 and Rht-D1 alleles.

- Selection of Rht8C + Rht-B1B or Rht-D1D could facilitate development of high-yielding varieties targeting favourable and unfavourable environments, such as drought and low N

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

- Lines generated to test this:
- *RhtB1* x *Rht8*
- *RhtD1* x *Rht8*
- *RhtB1* x *RhtD1*

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

- In addition a 'winter Paragon' was produced by crossing in *VrnA1* + *VrnB1* from Malacca
- Subsequently crossed into *Rht8* / *RhtB1* / *RhtD1* to produce a winter, semi-dwarf, Paragon

WGIN3 Projects: Avalon and Cadenza

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WGIN3 Projects: Avalon and Cadenza

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- WGIN successfully promoted the AxC 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

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

5. Understanding genotype x environment interaction in Avalon x Cadenza

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

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

- The BC3 NILs carry selected genetic foreground in the QTL regions (height, heading, and yield)
- In addition each line carries ~12.5% random chromosomal background.
- Plan to tile the whole genome with chromosome segments to make recombinant substitution lines for genome.

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

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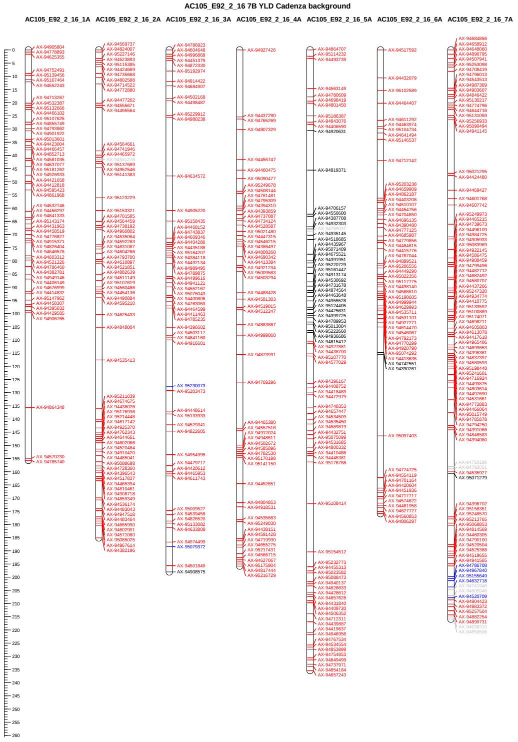
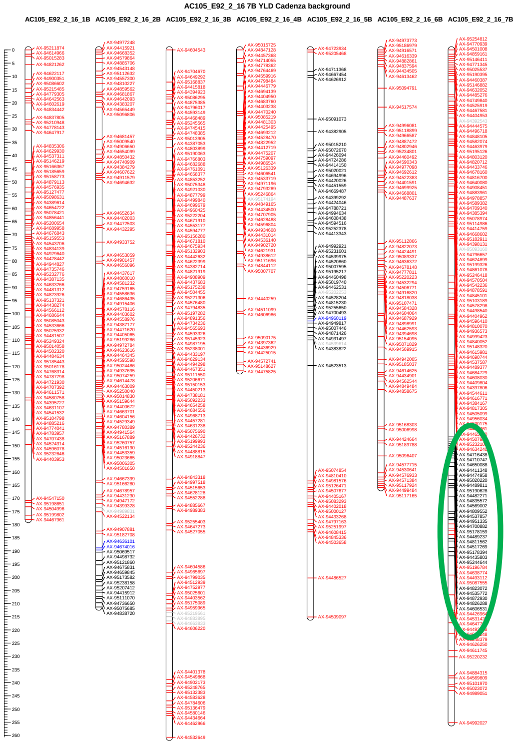
- Preliminary experiment 18 NILs and 820K array
- Maps based on Bristol Frame-Map

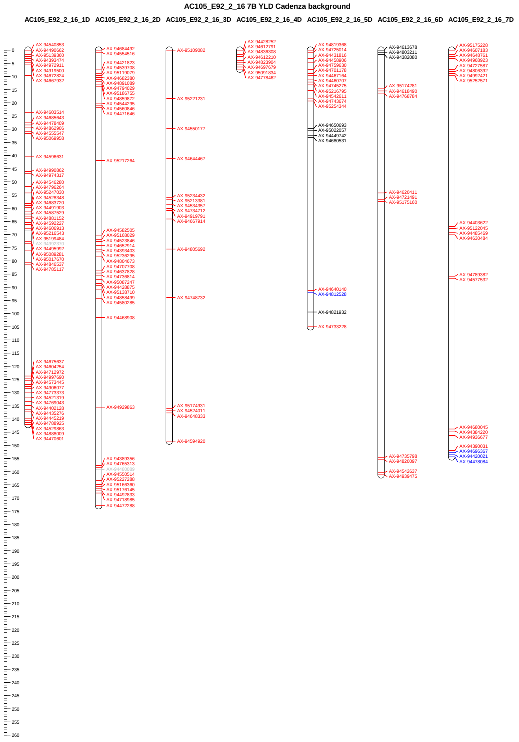
A Genome



B Genome



D Genome



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

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

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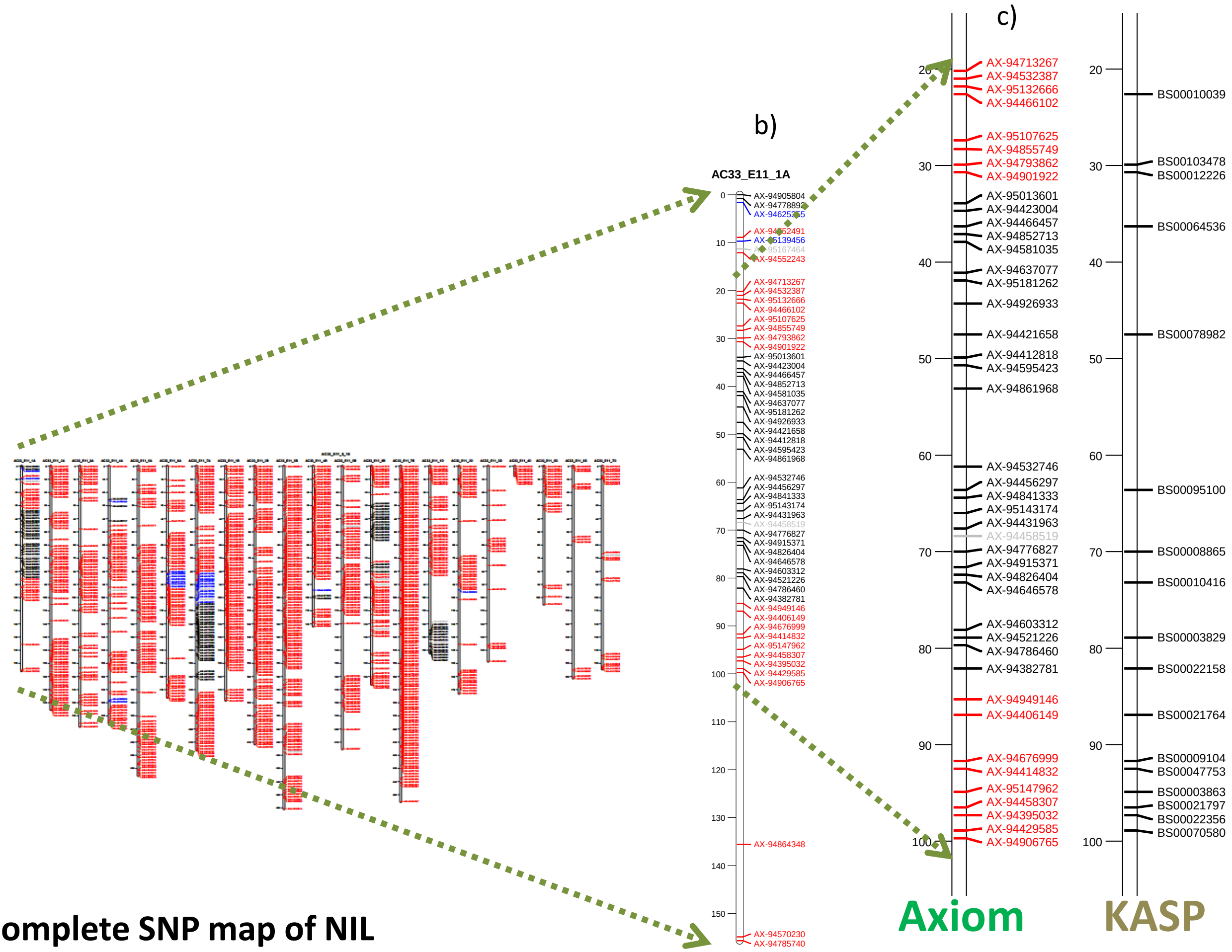

Complete SNP map of NIL
With QTL for 1D DTEM

Chromosome 1A

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

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

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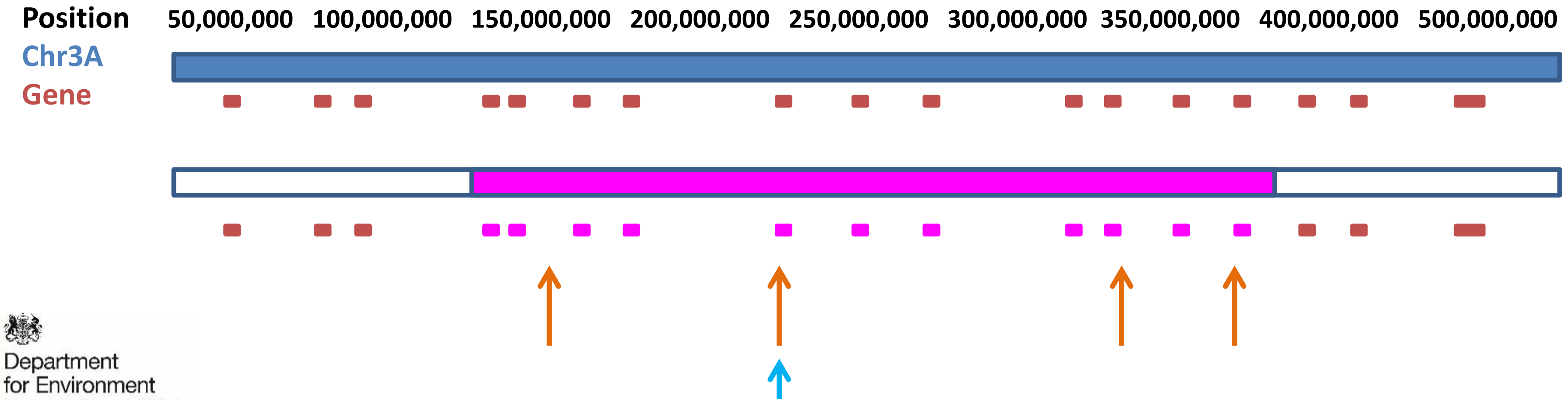
- Publication and availability of genomic sequence will allow more sophisticated presentation of these data in future
- Possible to present these in an *interactive* format
- Include as part of a searchable genome browser.
- Indication extent of substituted segment.
- Show position of Axiom ↑ and Bristol ↑ markers



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

- Genotyping carried out on 94 NILs (+ Av/Cad) using Breeders 35K array
- Estimate of % coverage of background (+ foreground) for each chromosome

- **Have used several methods to generate map (i.e. based on frame-map, Axiom info and pseudomolecules…)**

- NILs backcrossed to the recurrent parent to generate lines where QTL region and random segments have been separated

	Avalon	**Cadenza**
	43 lines	**46 lines**
1A	~80%	~60%
1B	~95%	~90%
1D	~30%	~70%
2A	~80%	~95%
2B	~60%	~90%
2D	~95%	~70%
3A	~95%	~95%
3B	~70%	~95%
3D	~80%	~60%
4A	~80%	~75%
4B	~80%	~85%
4D	~80%	~95%
5A	~90%	~75%
5B	~80%	~90%
5D	~95%	~80%
6A	~95%	~85%
6B	~95%	~95%
6D	~95%	~95%
7A	~60%	~70%
7A or 7D	~40%	~90%
7B	~60%	~90%
7D	~95%	~30%

INCLUDES FOREGROUND

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5. Understanding genotype x environment interaction in Avalon x Cadenza

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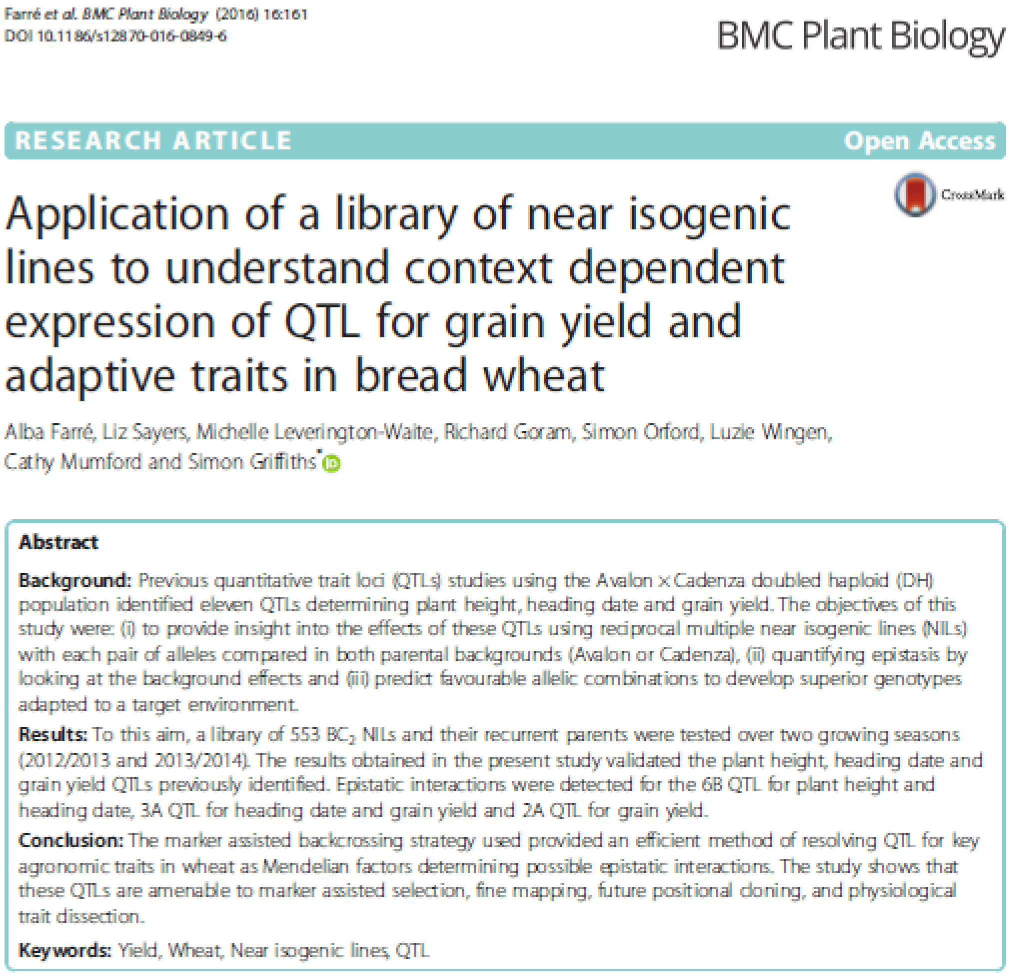

Farré *et al. BMC Plant Biology* (2016) 16:161
DOI 10.1186/s12870-016-0849-6

BMC Plant Biology

RESEARCH ARTICLE Open Access

CrossMark

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

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

Abstract

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

Results: To this aim, a library of 553 BC_2 NILs and their recurrent parents were tested over two growing seasons (2012/2013 and 2013/2014). The results obtained in the present study validated the plant height, heading date and grain yield QTLs previously identified. Epistatic interactions were detected for the 6B QTL for plant height and heading date, 3A QTL for heading date and grain yield and 2A QTL for grain yield.

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

Keywords: Yield, Wheat, Near isogenic lines, QTL

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

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

- All crosses at F2
- Populations in yellow at F3 (-> F5/F6) aiming for ~400 lines each
- * Population very sensitive to mildew (in glasshouse)
- * Will be sown for RAGT to follow up population

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

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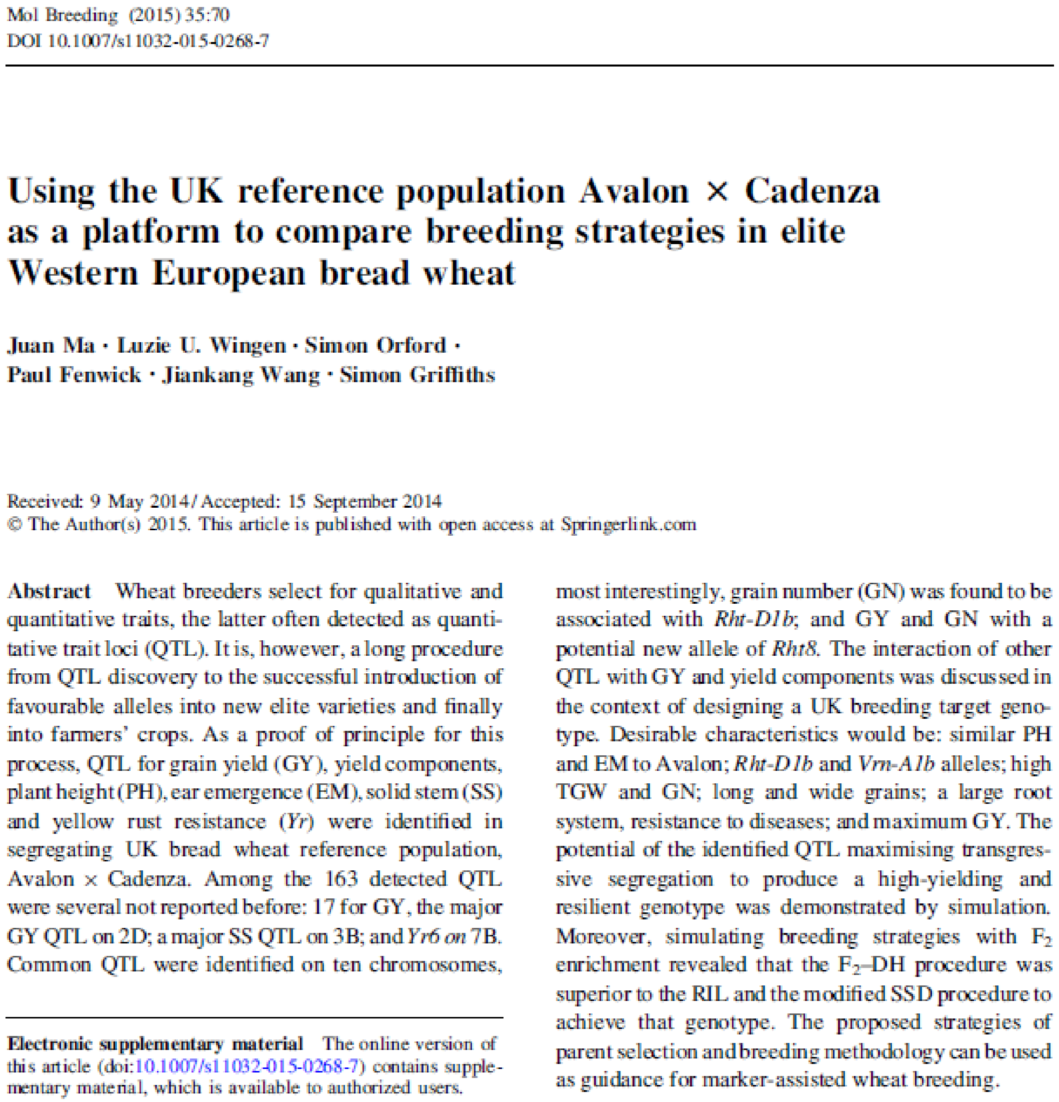

Mol Breeding (2015) 35:70
DOI 10.1007/s11032-015-0268-7

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

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

Received: 9 May 2014 / Accepted: 15 September 2014

Abstract Wheat breeders select for qualitative and quantitative traits, the latter often detected as quantitative trait loci (QTL). It is, however, a long procedure from QTL discovery to the successful introduction of favourable alleles into new elite varieties and finally into farmers' crops. As a proof of principle for this process, QTL for grain yield (GY), yield components, plant height (PH), ear emergence (EM), solid stem (SS) and yellow rust resistance (*Yr*) were identified in segregating UK bread wheat reference population, Avalon × Cadenza. Among the 163 detected QTL were several not reported before: 17 for GY, the major GY QTL on 2D; a major SS QTL on 3B; and *Yr6 on* 7B. Common QTL were identified on ten chromosomes, most interestingly, grain number (GN) was found to be associated with *Rht-D1b*, and GY and GN with a potential new allele of *Rht8*. The interaction of other QTL with GY and yield components was discussed in the context of designing a UK breeding target genotype. Desirable characteristics would be: similar PH and EM to Avalon; *Rht-D1b* and *Vrn-A1b* alleles; high TGW and GN; long and wide grains; a large root system, resistance to diseases; and maximum GY. The potential of the identified QTL maximising transgressive segregation to produce a high-yielding and resilient genotype was demonstrated by simulation. Moreover, simulating breeding strategies with F_2 enrichment revealed that the F_2–DH procedure was superior to the RIL and the modified SSD procedure to achieve that genotype. The proposed strategies of parent selection and breeding methodology can be used as guidance for marker-assisted wheat breeding.

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

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

- ‘Ideal’ ACDH lines to be crossed were selected by analysis of the QTL data
- These would have three high yielding QTL (GY) alleles

- However most ‘ideal’ lines had unfavourable QTL alleles elsewhere
- Therefore made best selection possible…

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

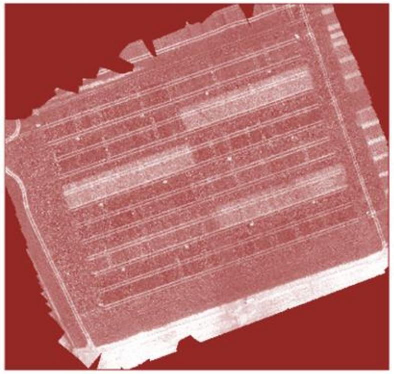
- F2 seed is being generated but few F1 seed…

WGIN3 Projects: Additional Populations

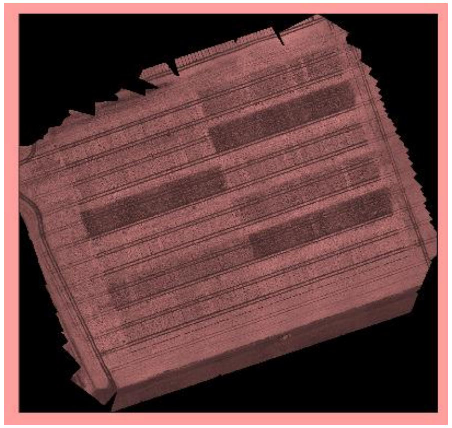
- 3N alien introgression (from *Aegilops uniaristata*) into Chinese Spring
- Shows aluminium tolerance
- Al toxicity primarily affects the division and elongation of the root apex
- 3N line shows prolific root phenotype.
- Crossed to winter elite lines – **Cordiale**, Napier and Robigus
- Homozygous lines being bulked for autumn sowing

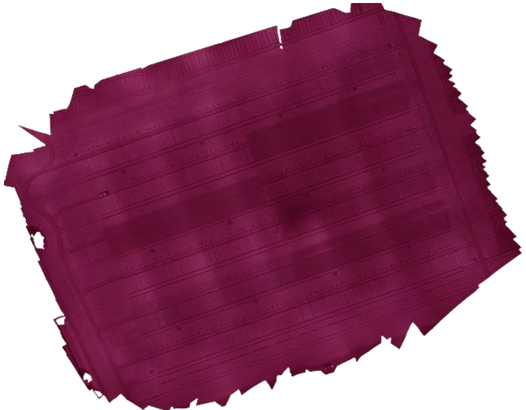
Rothamsted Research
where knowledge grows

Using the WGIN Diversity trial to develop drone applications



Andrew Riche





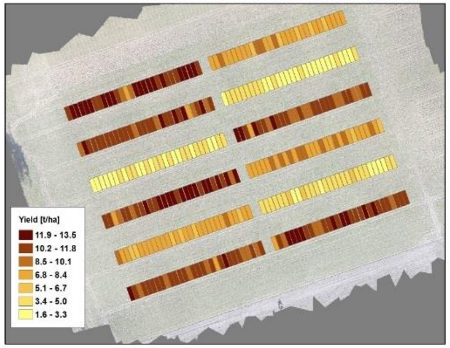


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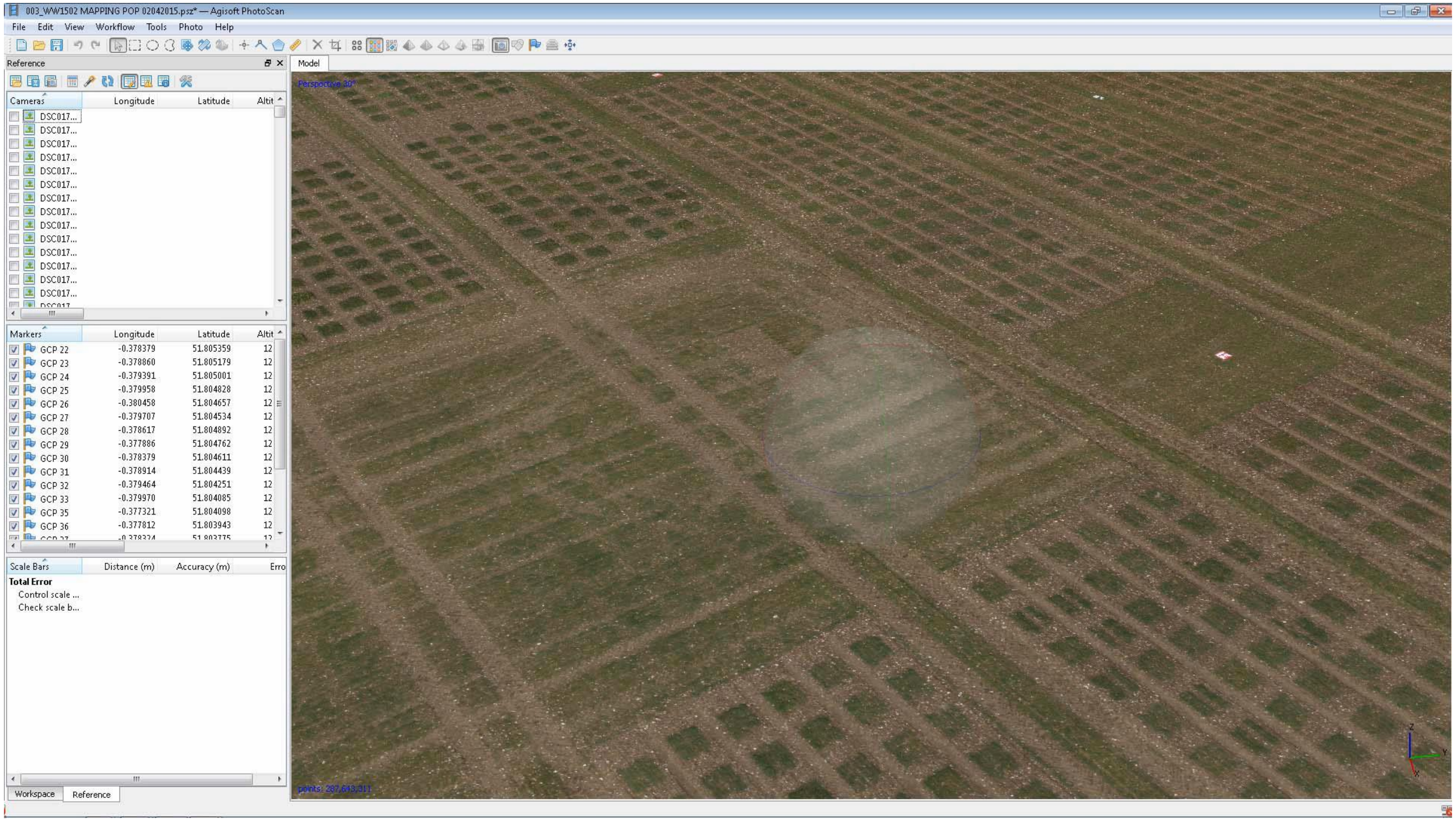
Image collection



Cameras	Longitude	Latitude	Altit
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☑ GCP 25	-0.379958	51.804828	12
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☑ GCP 27	-0.379707	51.804534	12
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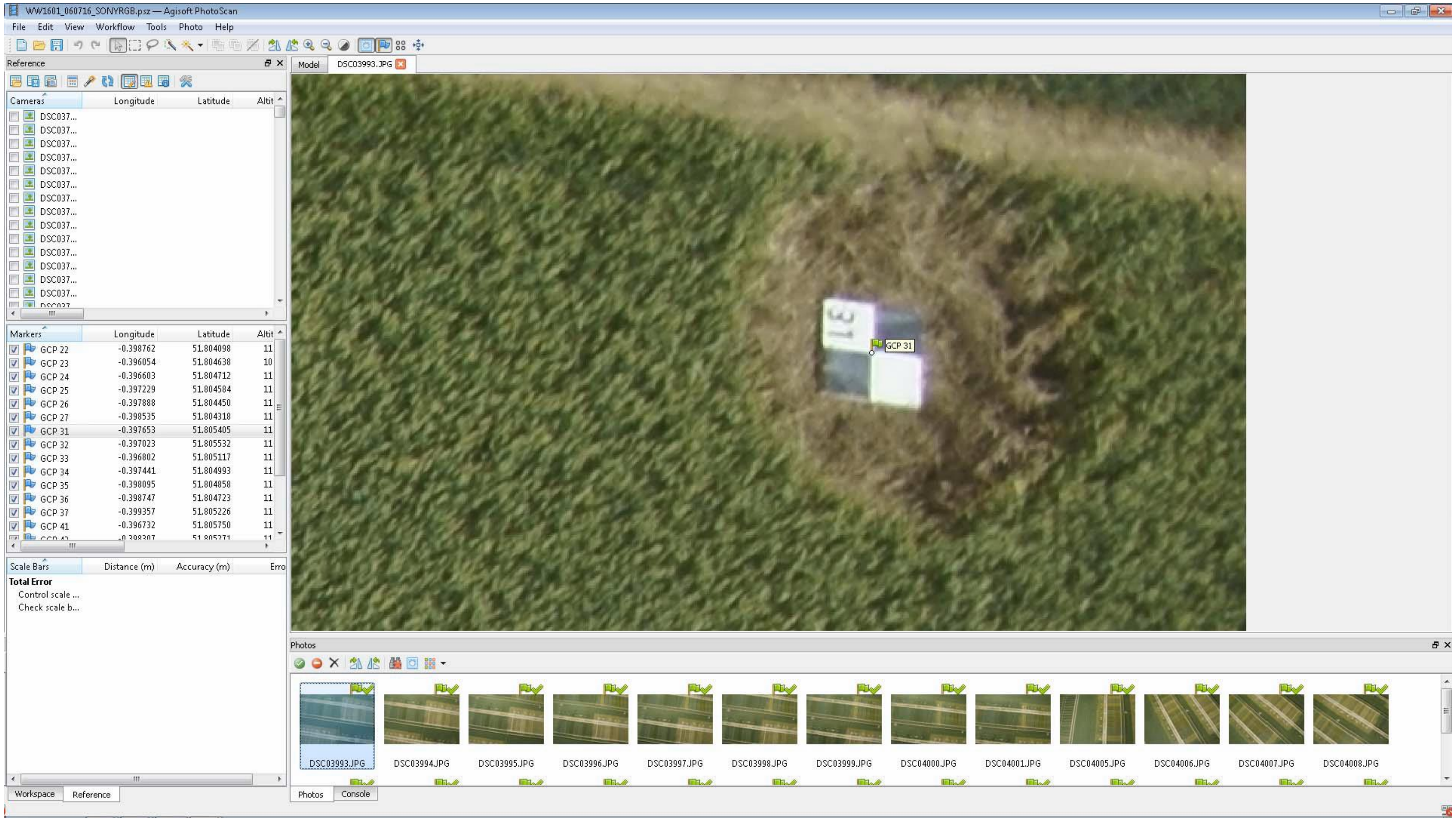
☑ GCP 35	-0.377321	51.804098	12
☑ GCP 36	-0.377812	51.803943	12

Scale Bars	Distance (m)	Accuracy (m)	Erro
Total Error			
Control scale ...			
Check scale b...			

Ground control points



Markers	Longitude	Latitude	Altit
☑ GCP 22	-0.398762	51.804098	11
☑ GCP 23	-0.396054	51.804638	10
☑ GCP 24	-0.396603	51.804712	11
☑ GCP 25	-0.397229	51.804584	11
☑ GCP 26	-0.397888	51.804450	11
☑ GCP 27	-0.398535	51.804318	11
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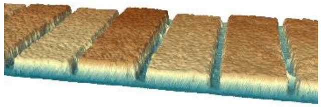
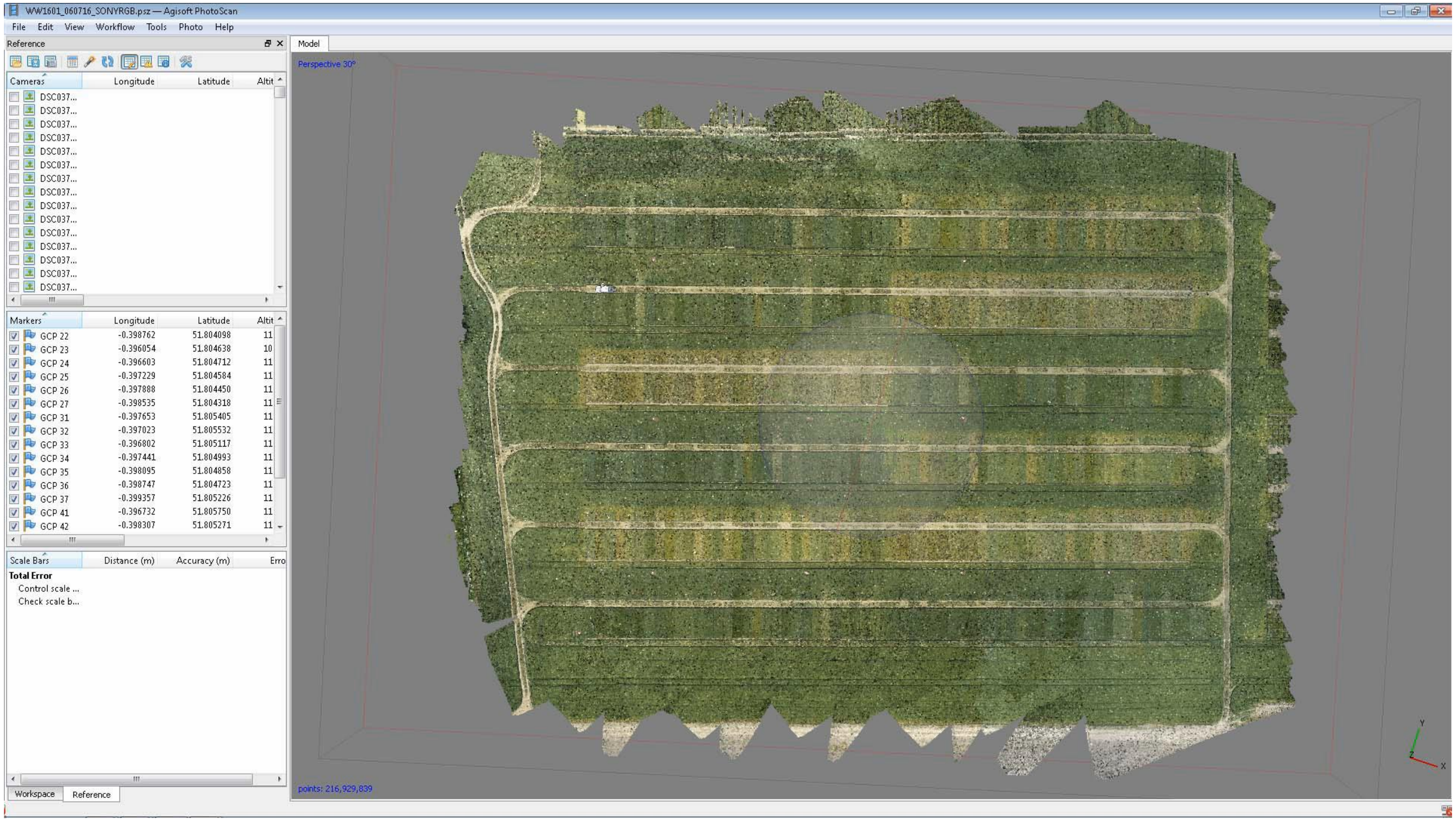
☑ GCP 33	-0.396802	51.805117	11
☑ GCP 34	-0.397441	51.804993	11
☑ GCP 35	-0.398095	51.804858	11
☑ GCP 36	-0.398747	51.804723	11
☑ GCP 37	-0.399357	51.805226	11
☑ GCP 41	-0.396732	51.805750	11

Output- point cloud





Cameras	Longitude	Latitude	Altit
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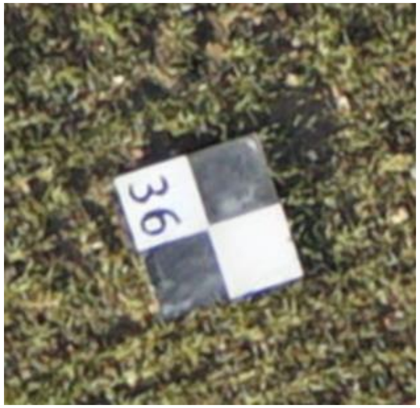
☑ GCP 41	-0.396732	51.805750	11
☑ GCP 42	-0.398307	51.805271	11

Scale Bars	Distance (m)	Accuracy (m)	Erro
Total Error			
Control scale ...			
Check scale b...			

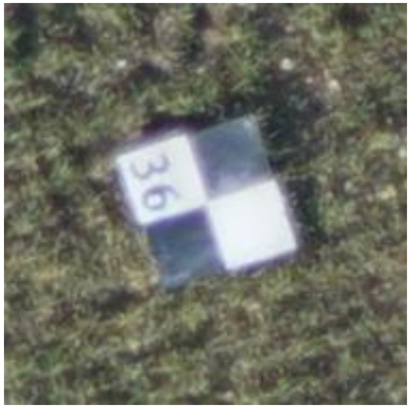
points: 216,929,839

Image quality - ISO

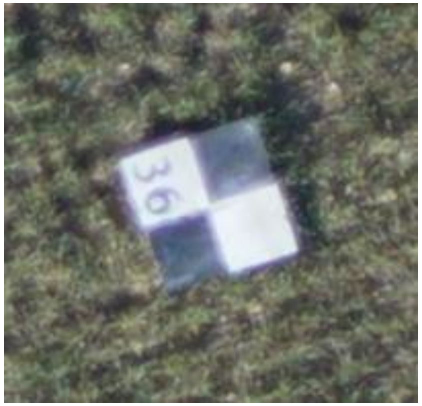


100 (f4.5)

125 (f2.8)



160



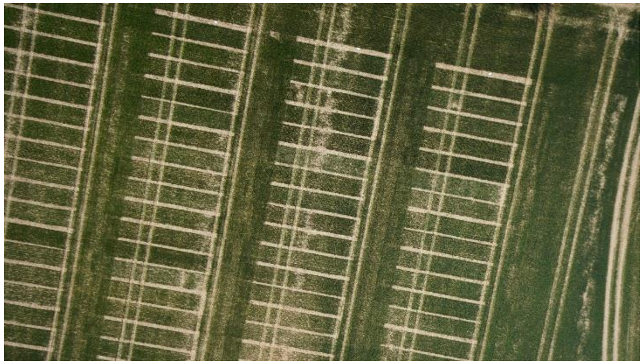
250

400

800

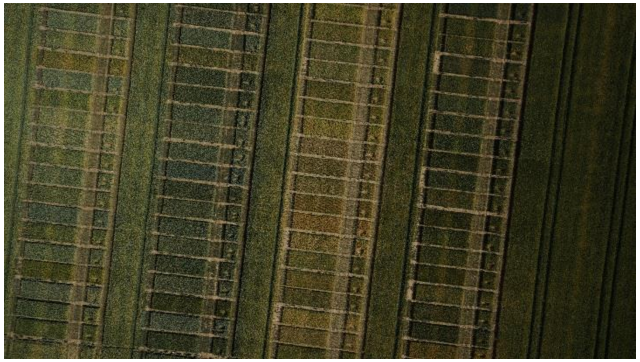
Effect on exposure of lack of brightness

10/4/14, 1/1250, f4.5 ISO 100



1/800, f4.5, ISO 100
2/3 stop slower

3/7/14, 1/500, f8.0, ISO 100

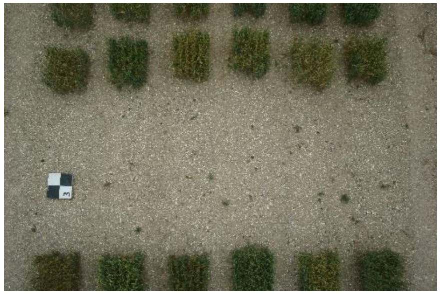


1/640, 7.1, ISO 100, exp comp -1
1 stop slower

Effect of Altitude on resolution

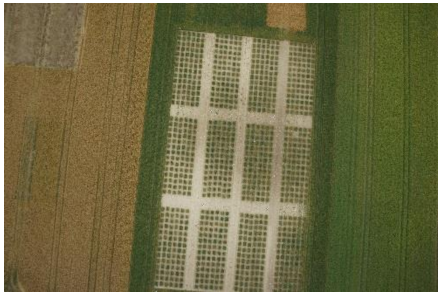
10m   2mm/pix

ISO 100, f7.1, 1/500



120m   23mm

ISO 100, F5.0, 1/500

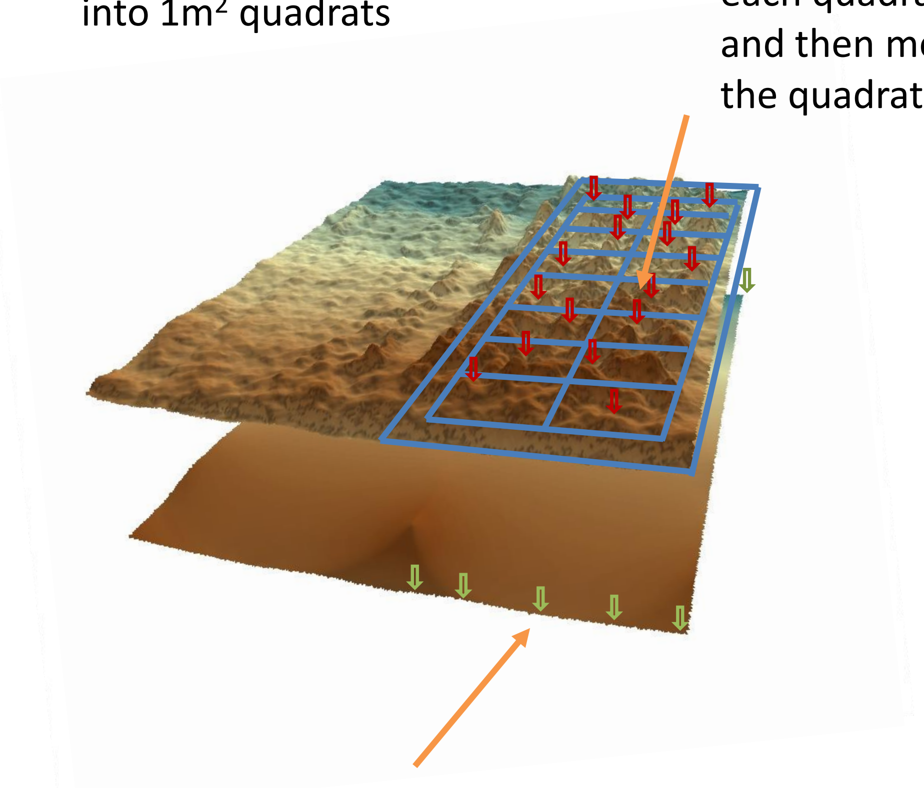


All with Sony 5100, 20mm lens, not mosaiced

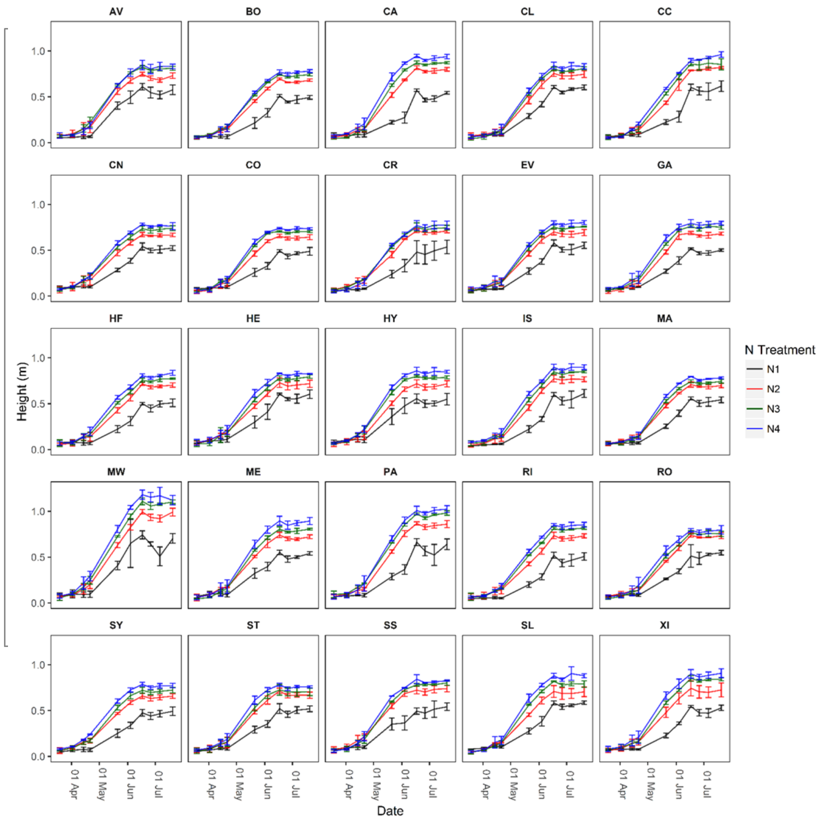
Measuring growth – Crop height

Centre of plot divided into 1m^2 quadrats

Maximum height within each quadrat extracted and then meaned across the quadrats



Ground height data extracted from bare ground border adjacent to plot, meaned across several data points

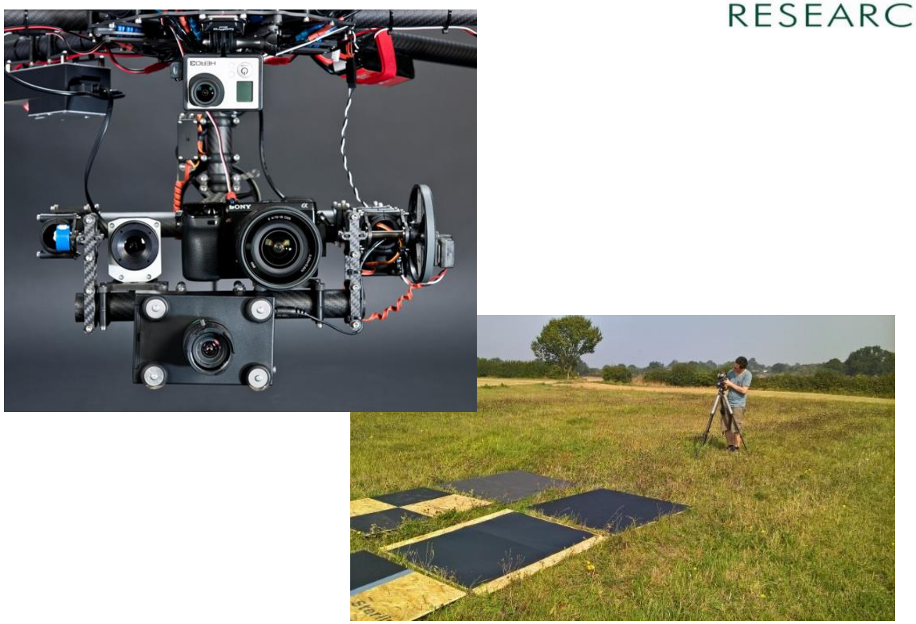


Thermal image processing - workflow

- Extract csv files from video
- Convert to integer values (x1000)
- Convert to tif files
- Correct all images for vignetting
- Create orthomosaic
- Extract data (/1000)
- Atmospheric correction

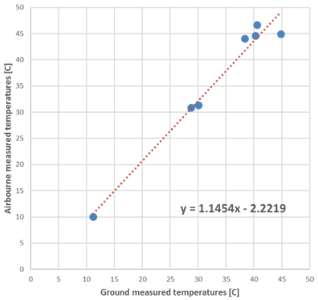
Also measured:

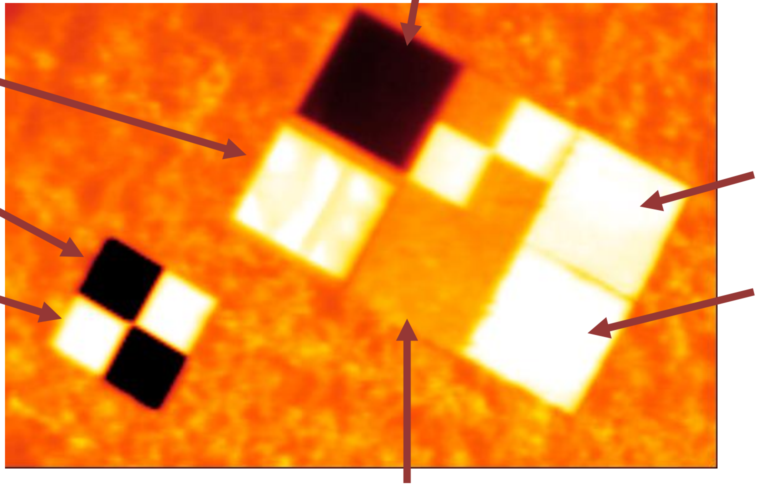
- Temp of calibration targets
- windspeed
- Air temperature at ground and on UAV
- [light level]



Thermal image processing - calibration

Ground control point – aluminium foil and black paint on wood





Polystyrene 10^{0}C

Black card 45^{0}C

Aluminium -20^{0}C

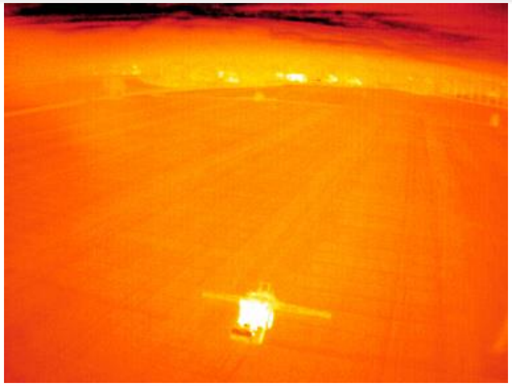
Black wood 45^{0}C

Bituminous roof felt 45^{0}C

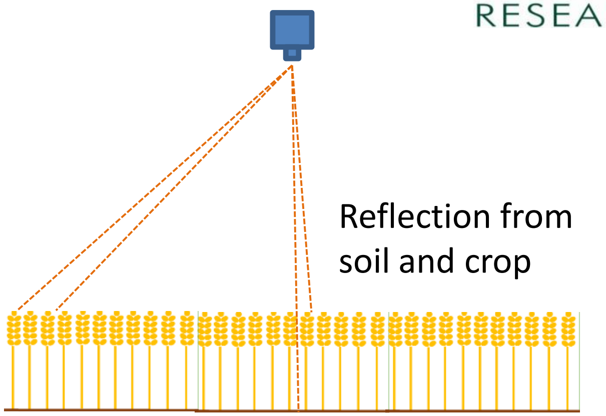
Black wood 46^{0}C

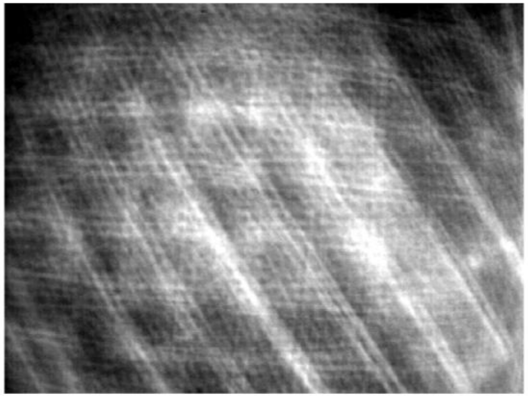
Wooden board 31^{0}C

Thermal image pre-processing - vignetting

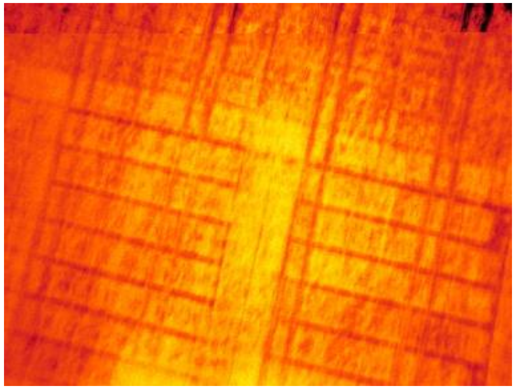


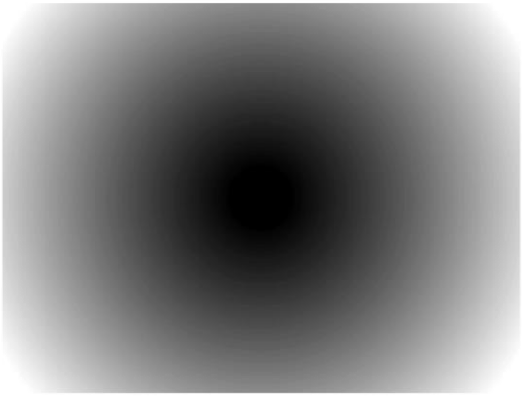
Two sources – real (optical) and apparent



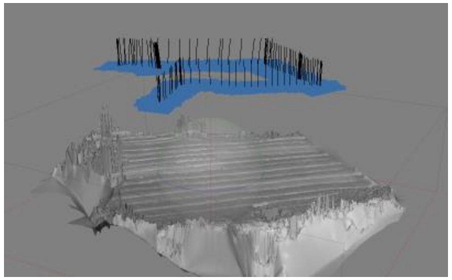


Stack of all images

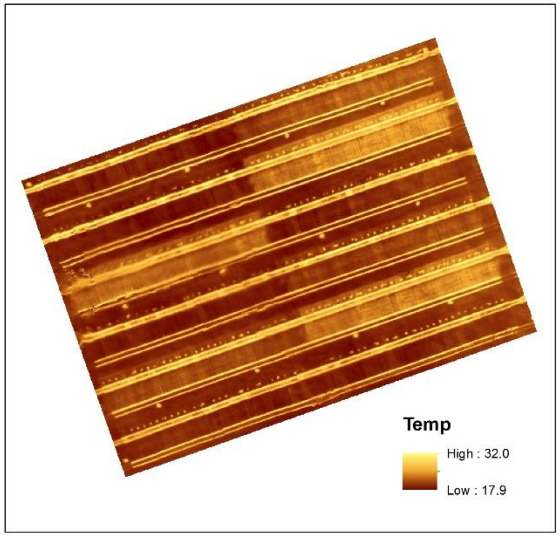


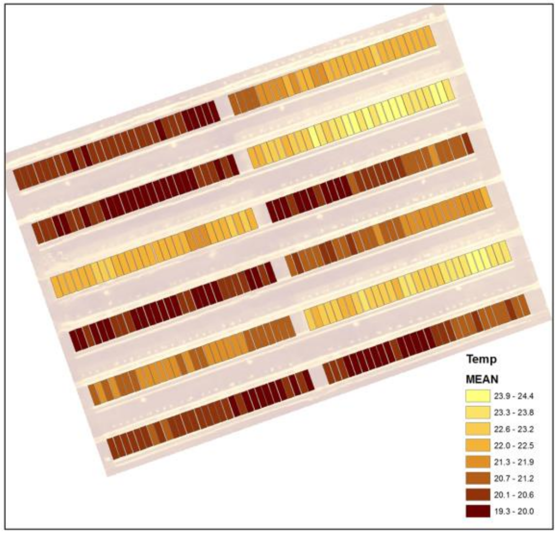


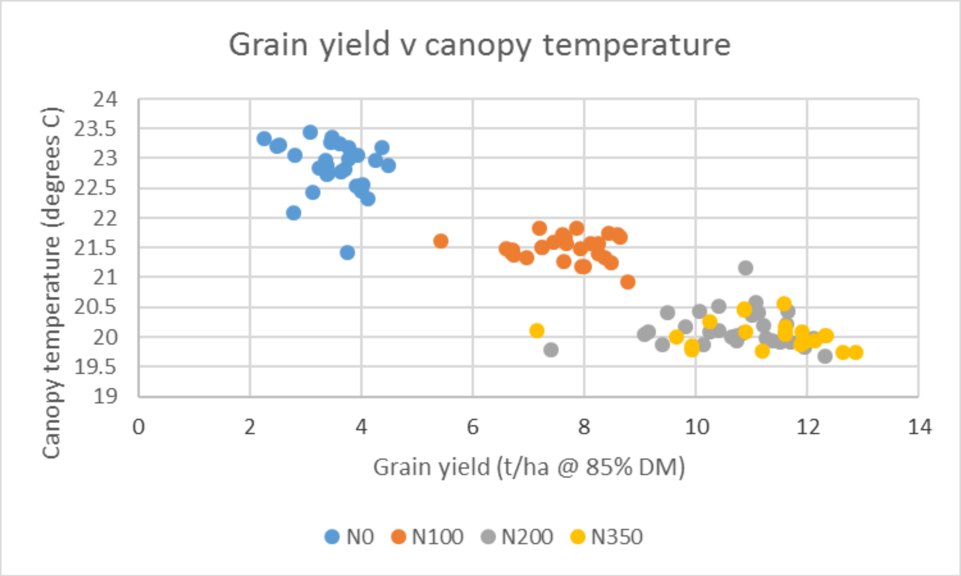
Filter



Thermal image processing – data extraction



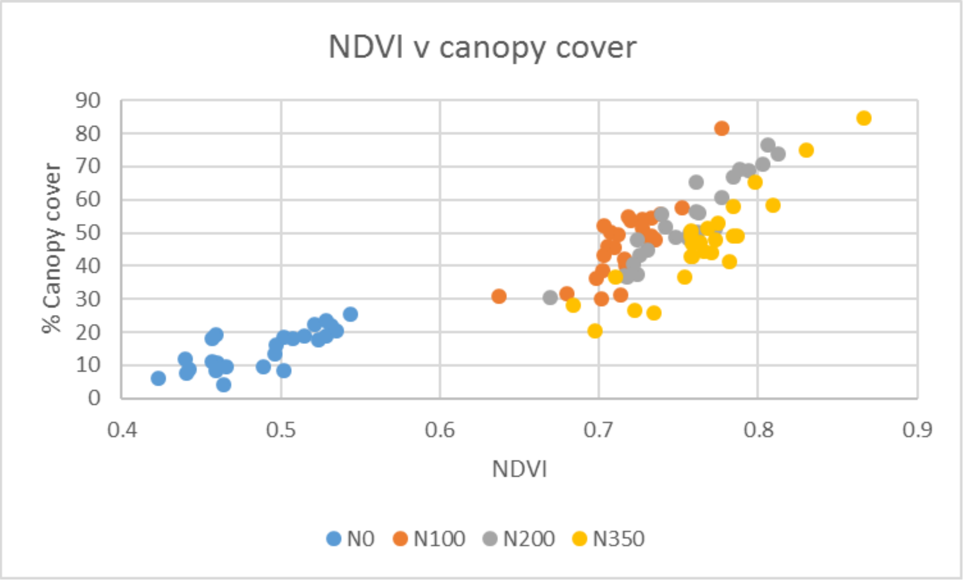


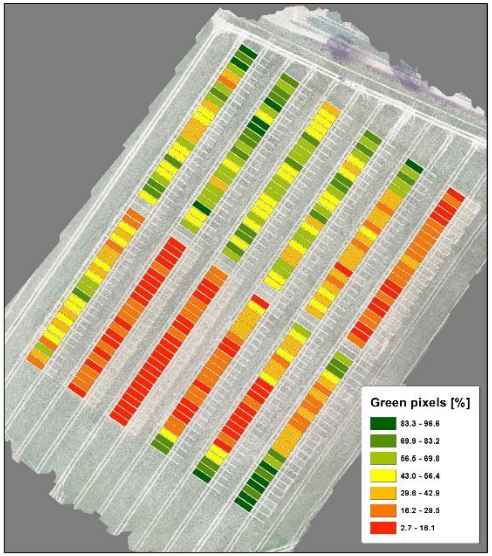


Vegetation classification – measuring canopy cover

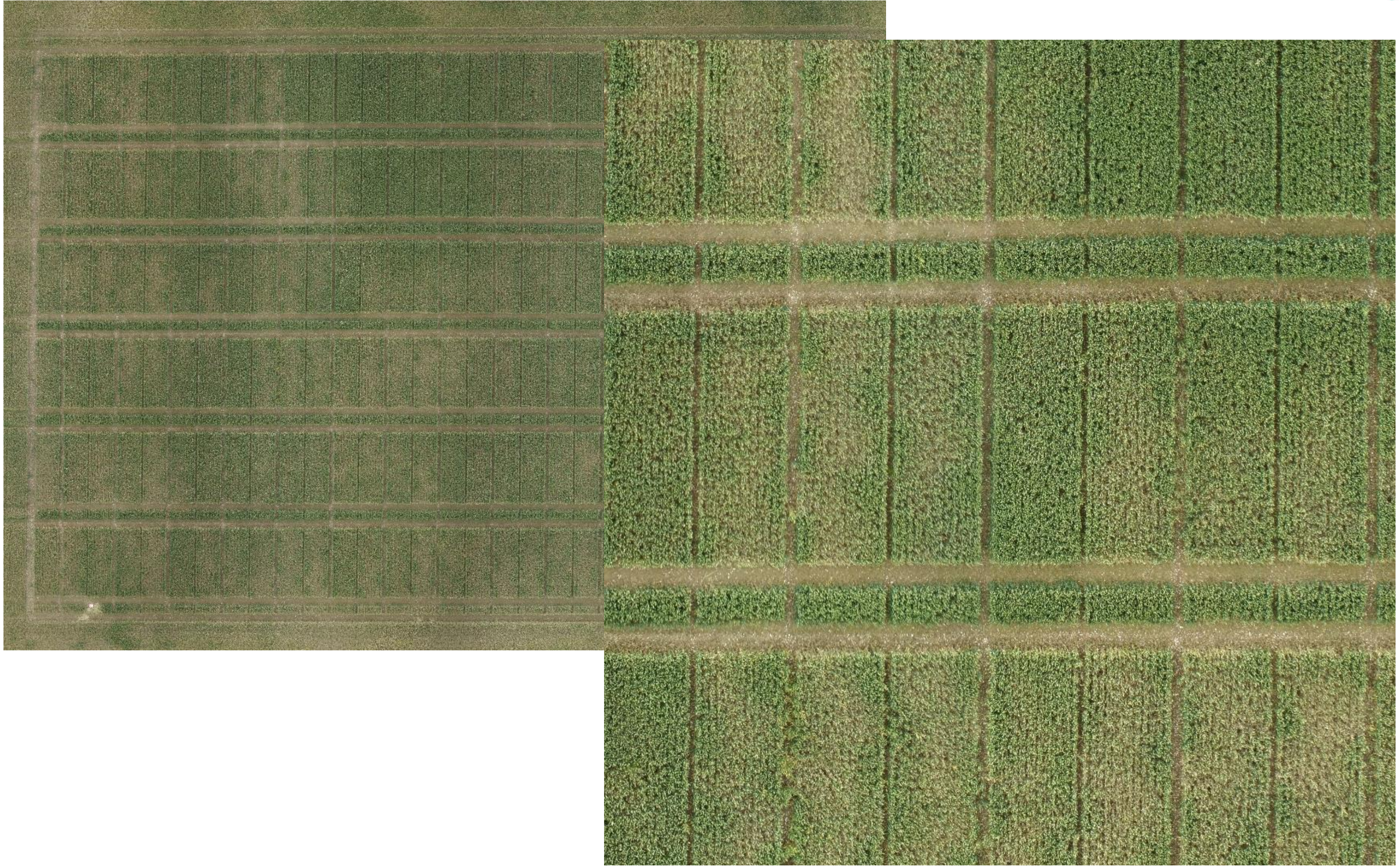
Diversity 08/04/15

ExGR (Excess green – excess red)
ExGR = (2xG-R-B) – (1.4 x R-G)





Vegetation classification – take-all



NDVI Workflow

Raw Photo

↓

Raw conversion

↓

Correct vignetting

↓

Correct exposure

↓

Correct for ambient light

↓

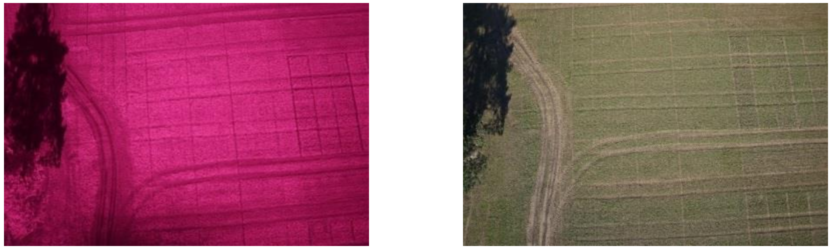
Mosaic – in Agisoft

Overlay Plot shape file - ArcMap

↓

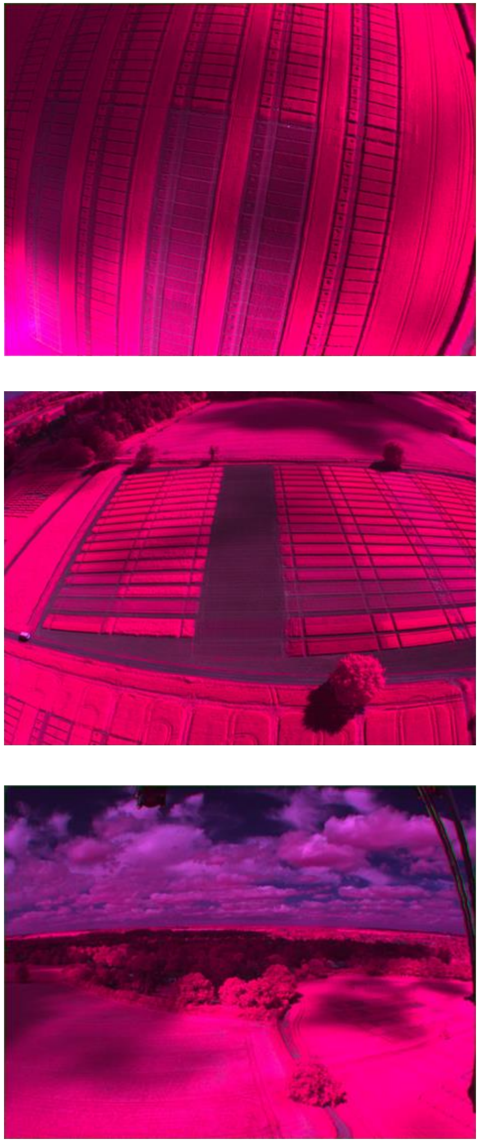
Extract NDVI data ArcMap

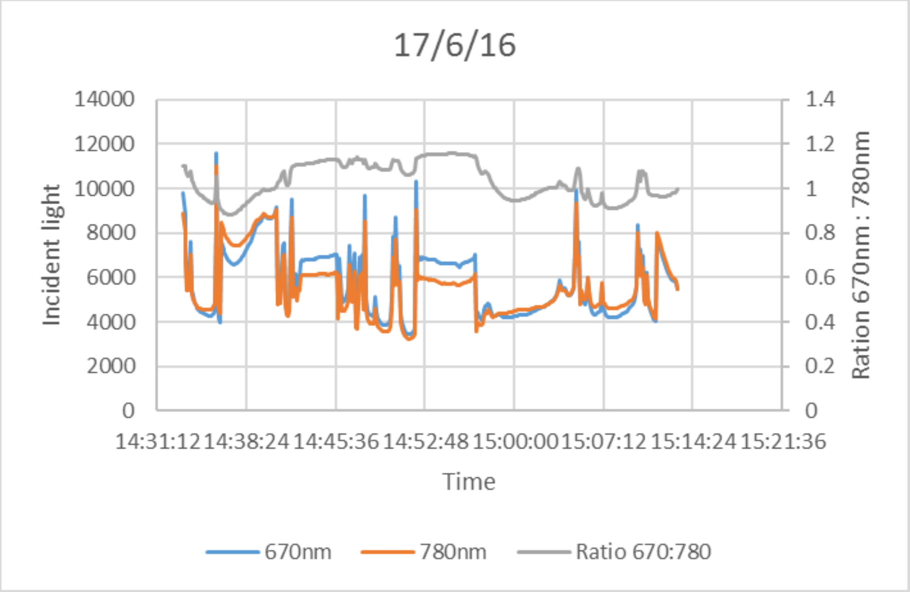
Workflow needs following for both RGB and NIR images



NDVI = (NIR – visible Red) / (NIR + visRed)

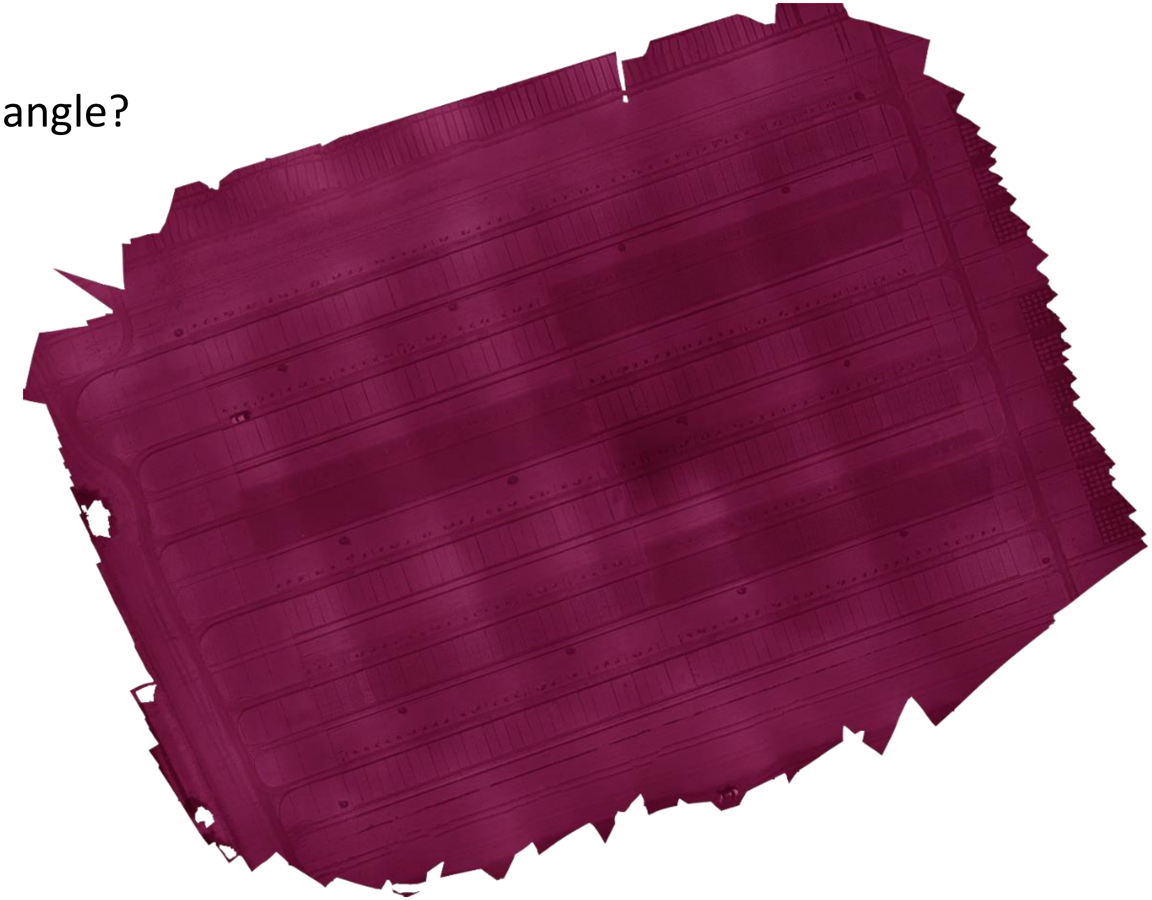
Variable light conditions



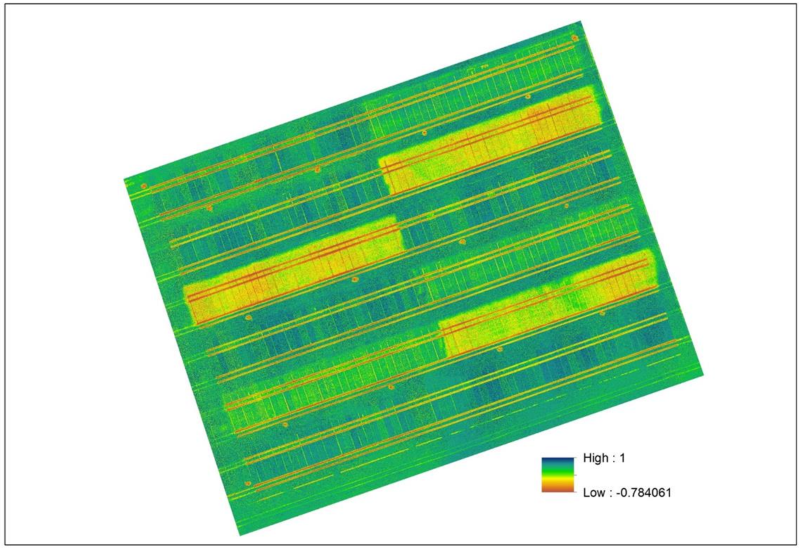


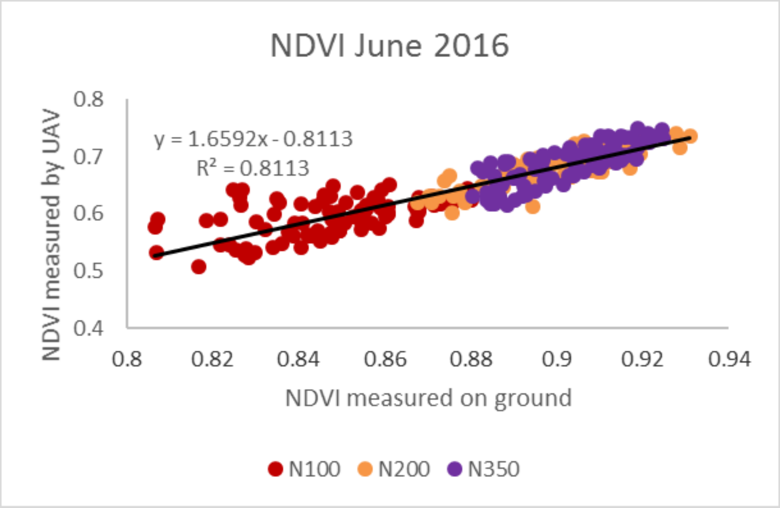
Issues with colour extraction

Banding – caused by camera angle?



NDVI


High : 1
Low : -0.784061


NDVI June 2016
y = 1.6592x - 0.8113
R² = 0.8113
NDVI measured by UAV
0.8
0.7
0.6
0.5
0.4
0.8
0.82
0.84
0.86
0.88
0.9
0.92
0.94
NDVI measured on ground
● N100
● N200
● N350

Conclusions

Crop height – can be accurately measured by UAV, workflow in place

Canopy temperature – can be measured by UAV, workflow in place

Canopy/green cover – can be measured by UAV, needs testing and workflow automating

Crop Indices – can be measured by UAV, image pre-processing needs developing

Lodging can be identified and estimated – method needs automating

Acknowledgements

March Castle

Adam Michalski

Also:
Malcolm Hawkesford
David Steele
Nicolas Virlet
Take-all team

Farm staff:
Stephen Goward
Chris Mackay
Nick Chichester-Miles

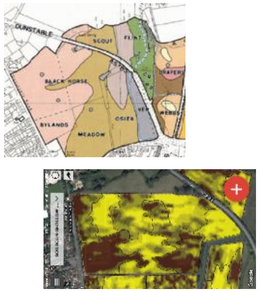
Kings Collage London:
Prof Martin Wooster
Fenner Holman

Utilizing spatial data

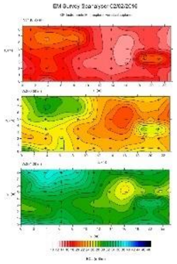
Spatial data

Co-factors:

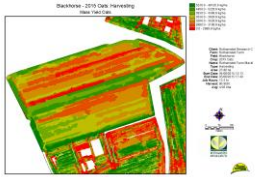
Soil type



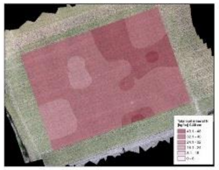
Soil EMI



Historical yield

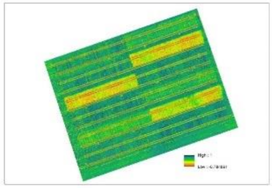


Soil N



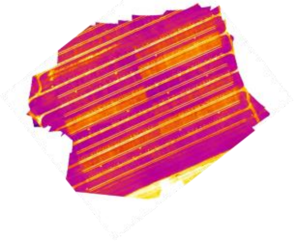
Experiment data

NDVI

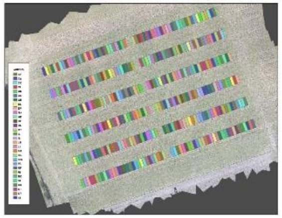


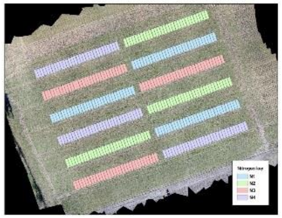
DSM/DEM

Canopy temp

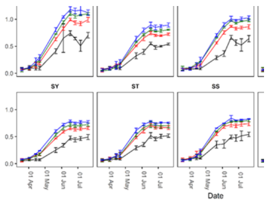


Overlay experimental layout

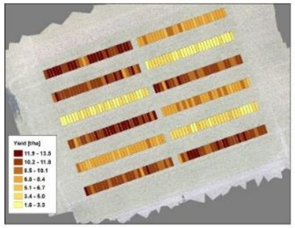


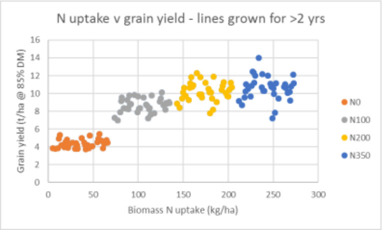


Extract data



For better interpretation of results





Rothamsted Research
where knowledge grows

WGIN3 Management Meeting

12th January 2017

Screening germplasm for resilience to aphids (WP2.3)

Lesley Smart

Department
for Environment
Food & Rural Affairs

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RESEARCH

Screening germplasm for resilience to aphids (WP2.3)

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

- Crosses of *T. monococcum* lines made by Mike Hammond-Kosack: MDR037 (susceptible) x MDR045, MDR049 and MDR657 (all showing evidence of partial resistance)

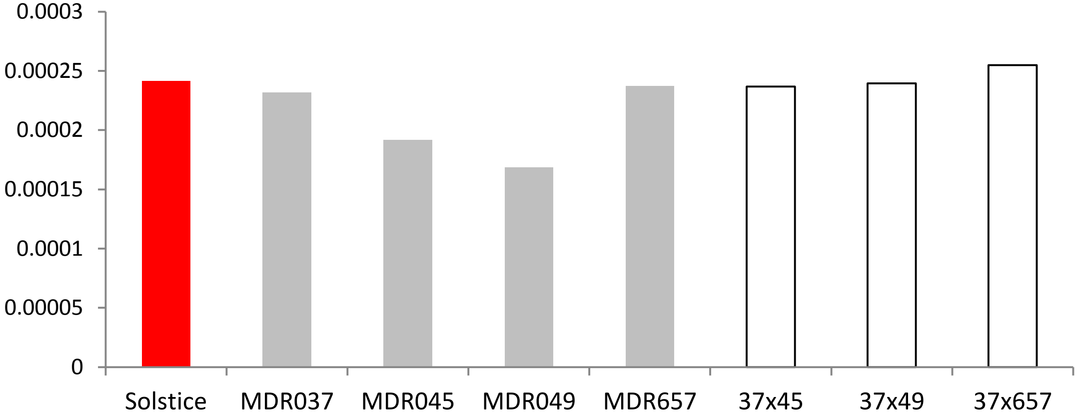
- F1 and F2 generations of these crosses have been tested in the phenotyping screen against both aphid species. MDR037 x MDR049 looked most promising.

- F3 generations of MDR037 x MDR049 are now being tested in the screen against both aphid species.

Screening germplasm for resilience to aphids (WP2.3)

Response to F1 generation of *T. monococcum* crosses

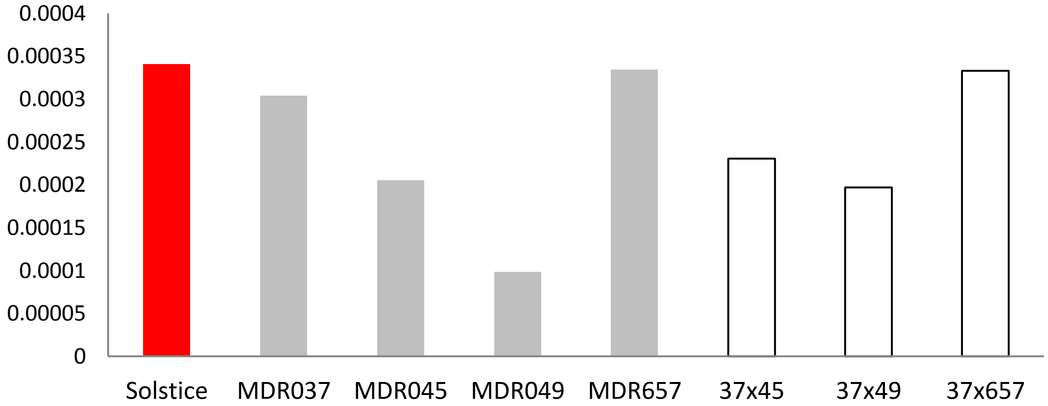
Rhopalosiphum padi **mean nymph weight (g) after 6 days on *T. monococcum*** parental lines **and F_1 crosses**



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

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

Sitobion avenae **mean nymph weight (g) 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

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

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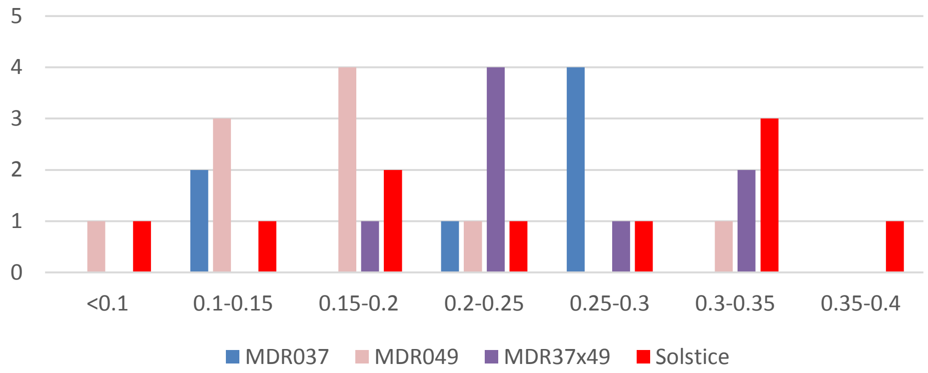
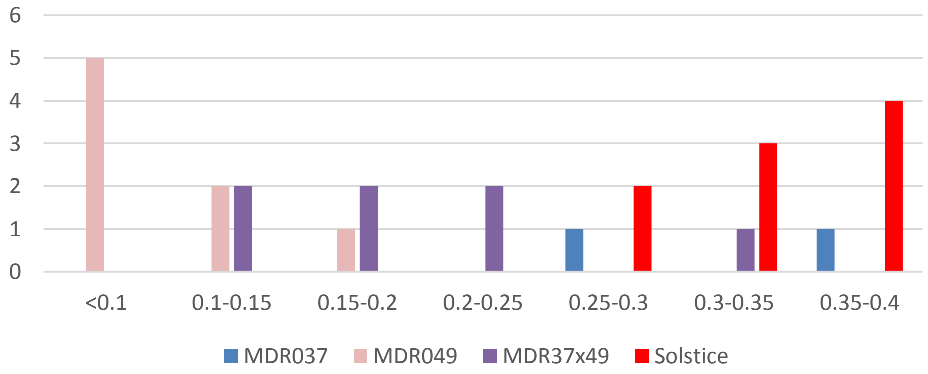
Screening germplasm for resilience to aphids (WP2.3)

Response to F1 generation of *T. monococcum* crosses of MDR037 x MDR049

R. padi on *T. monococcum* parental lines and MDR37 x 49 F1 crosses
no. nymphs per weight range at 6 days (mg)
n=7, 10, 8 & 10 respectively



S. avenae on *T. monococcum* parental lines and MDR37 x 49 F1 crosses
no. nymphs per weight range at 7 days (mg)
n=2, 8, 7 & 9 respectively



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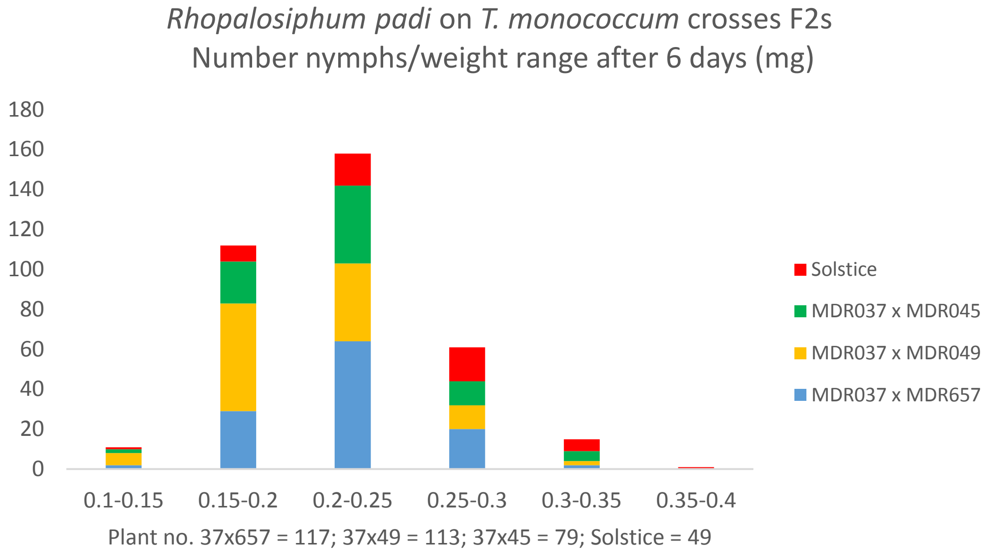
Screening germplasm for resilience to aphids (WP2.3)

Response to F2 generation of *T. monococcum* crosses

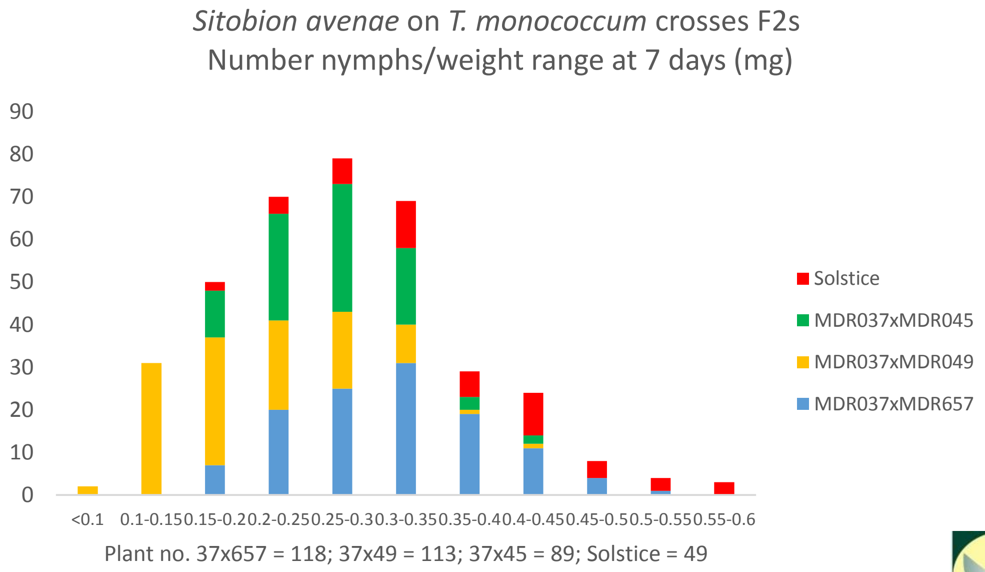


Rhopalosiphum padi on *T. monococcum* crosses F2s
Number nymphs/weight range after 6 days (mg)

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

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

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



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

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

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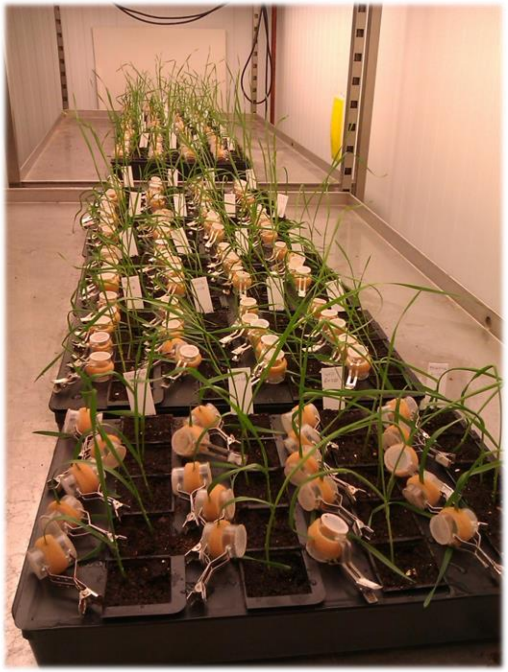
Screening germplasm for resilience to aphids (WP2.3)

Response to F3 generation of *T. monococcum* crosses **MDR037 x MDR049**



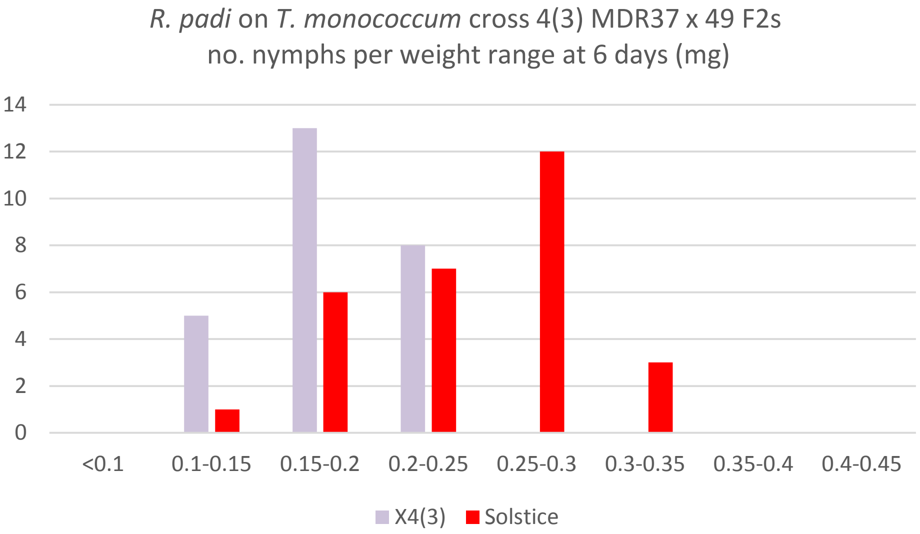
X4(3) MDR037 (1) x MDR049(1)
X5(1) MDR037 (1) x MDR049(2)
X11(3) MDR037 (1) x MDR049(1)
X17(1) MDR037 (2) x MDR049(1)

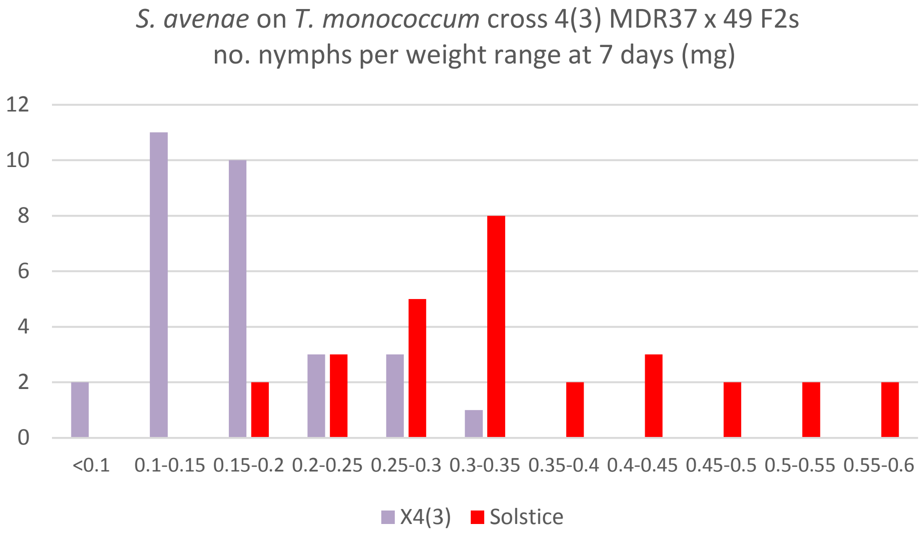
15 seeds of each selected F3 line sown and seedlings tested against target aphid species in phenotyping screen, compared to Solstice standard.

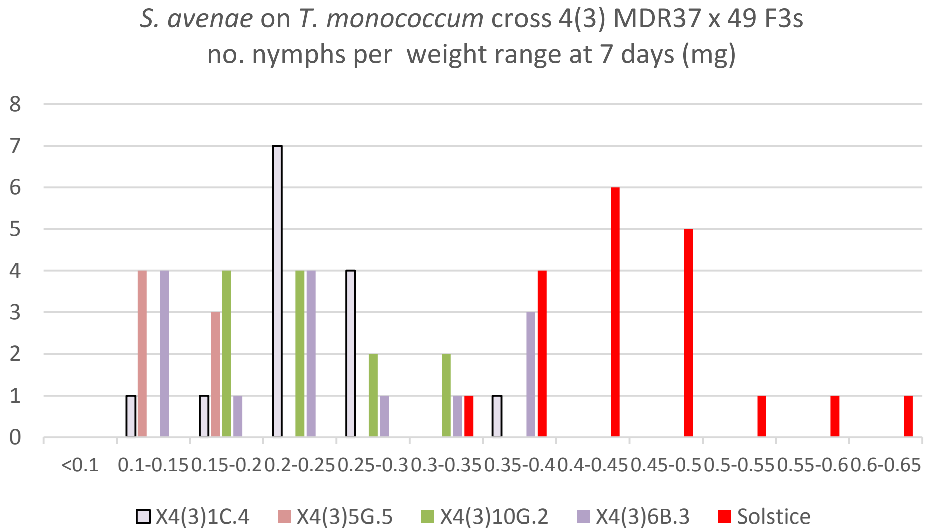
Number of surviving nymphs recorded and then weighed in their batches to get an average nymph weight. Weights then allocated to weight ranges for comparison.

Screening germplasm for resilience to aphids (WP2.3)

Response to F3 generation of *T. monococcum* cross X4(3) MDR037 x MDR049







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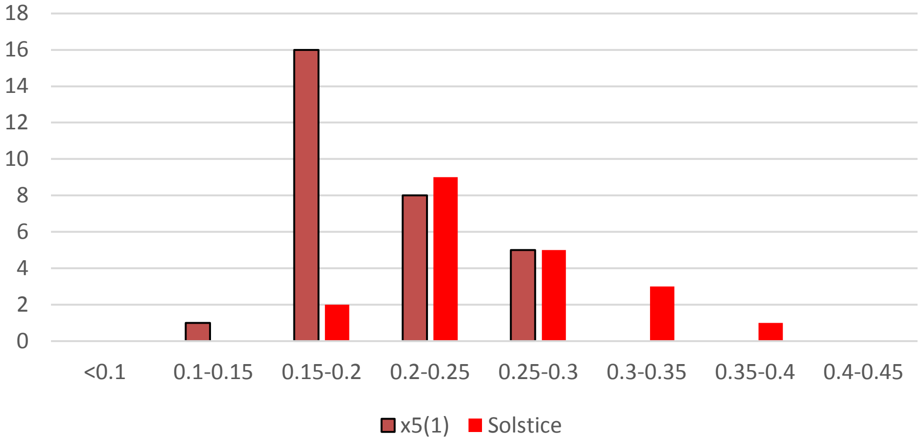
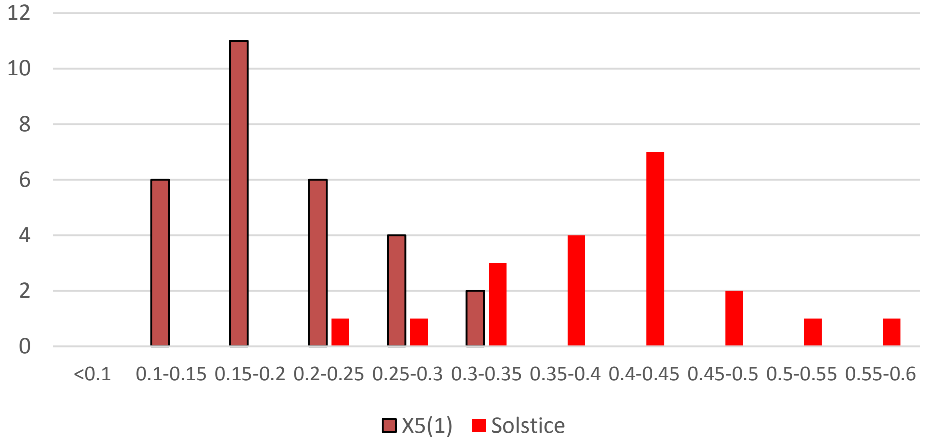
Screening germplasm for resilience to aphids (WP2.3)

Response to F3 generation of *T. monococcum* cross X5(1) MDR037 x MDR049

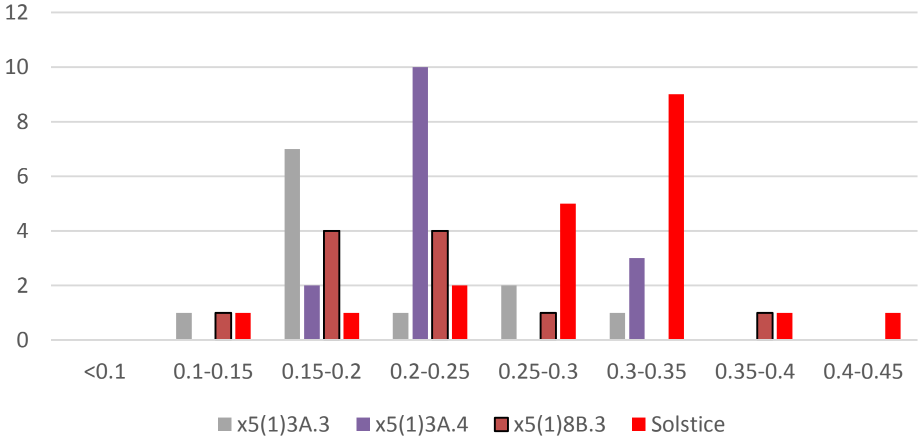
R. padi on *T. monococcum* cross 5(1) MDR37 x 49 F2s
no. nymphs per weight range at 6 days (mg)



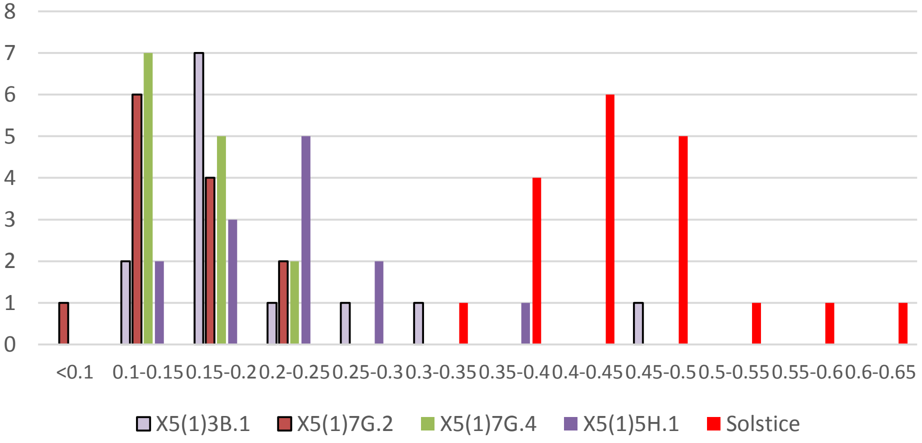
S. avenae on *T. monococcum* cross 5(1) MDR37 x 49 F2s
no. nymphs per weight range at 7 days (mg)



R. padi on *T. monococcum* cross 5(1) MDR37 x 49 F3s
no. nymphs per weight range at 6 days (mg)



S. avenae on *T. monococcum* cross 5(1) MDR37 x 49 F3s
no. nymphs per weight range at 7 days (mg)



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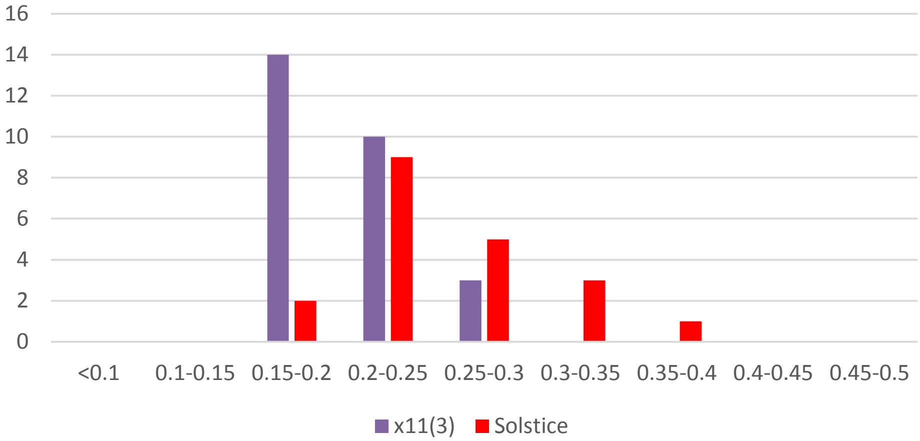
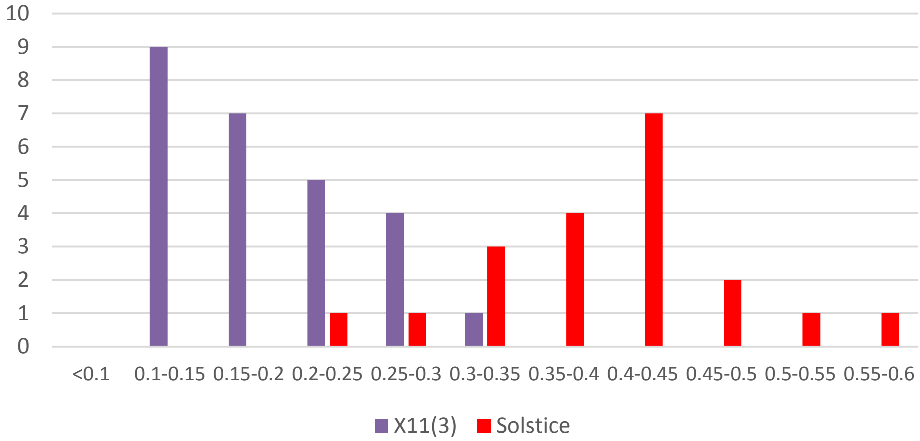
Screening germplasm for resilience to aphids (WP2.3)

Response to F3 generation of *T. monococcum* cross X11(3) MDR037 x MDR049

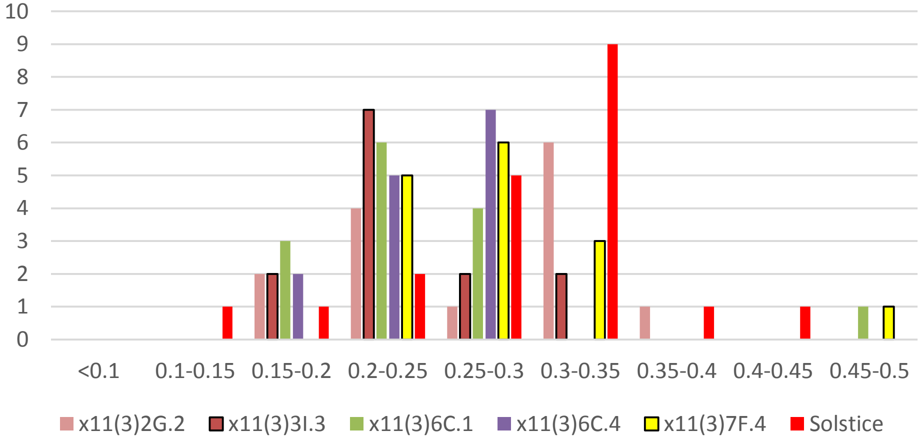
R. padi on *T. monococcum* cross 11(3) MDR37 x 49 F2s
no. nymphs per weight range at 6 days (mg)



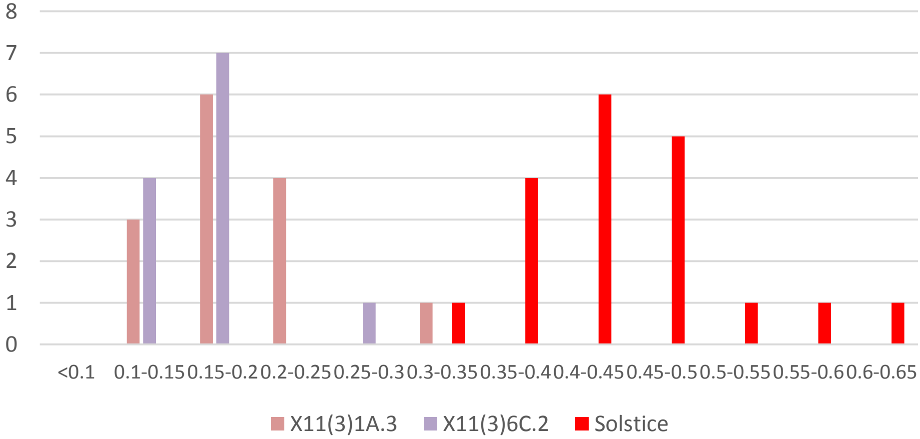
S. avenae on *T. monococcum* cross 11(3) MDR37 x 49 F2s
no. nymphs per weight range at 7 days (mg)



R. padi on *T. monococcum* cross 11(3) MDR37 x 49 F3s
no. nymphs per weight range at 6 days (mg)



S. avenae on *T. monococcum* cross 11(3) MDR37 x 49 F3s
no. nymphs per weight range at 7 days (mg)



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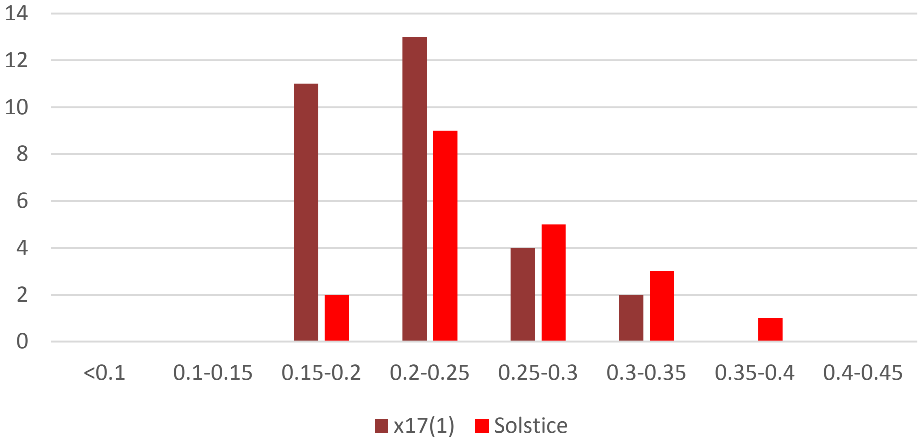
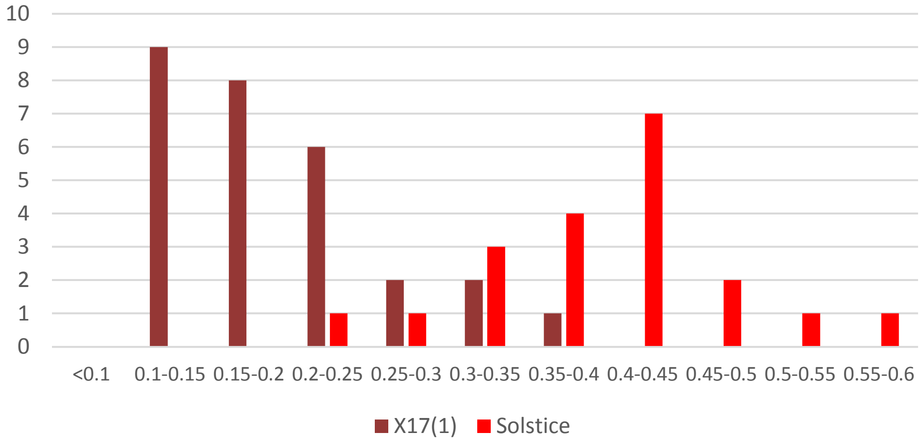
Screening germplasm for resilience to aphids (WP2.3)

Response to F3 generation of *T. monococcum* cross X17(1) MDR037 x MDR049

R. padi on *T. monococcum* cross 17(1) MDR37 x 49 F2s
no. nymphs per weight range at 6 days (mg)



S. avenae on *T. monococcum* cross 17(1) MDR37 x 49 F2s
no. nymphs per weight range at 7 days (mg)



R. padi on *T. monococcum* cross 17(1) MDR37 x 49 F3s
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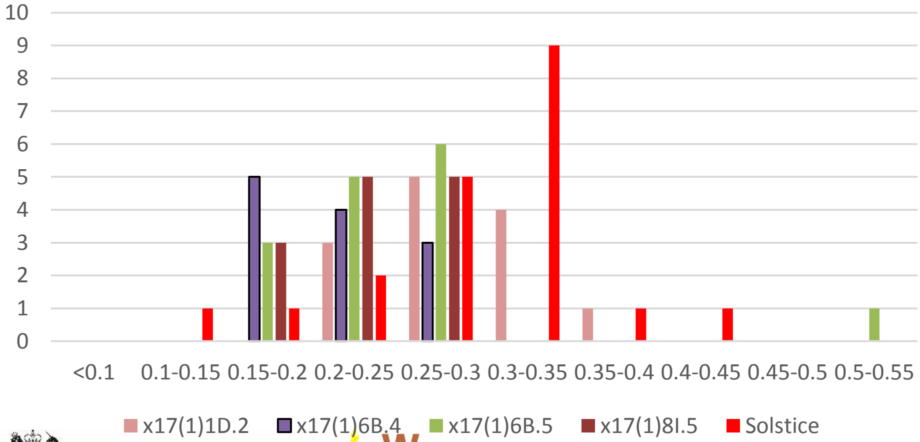

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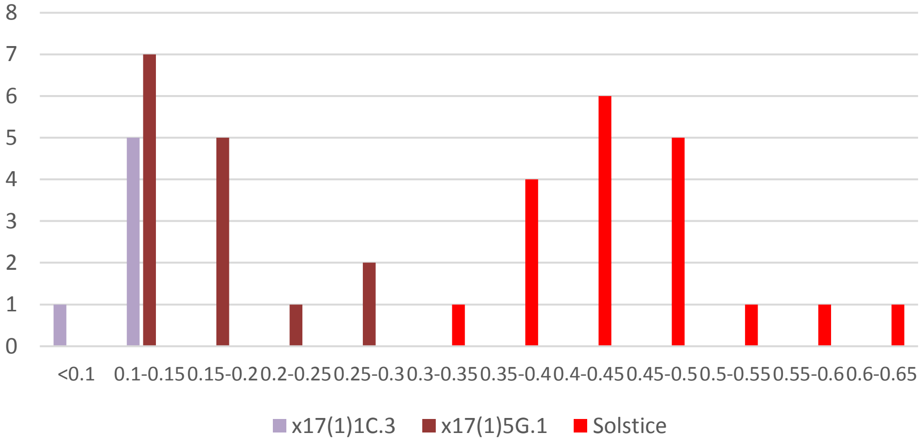
no. nymphs per weight range at 7 days (mg)



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Summary WGIN3 (WP2.3)

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- F3 generations of *T. monococcum* MDR037 x MDR049 crosses, with the best partial resistance and sufficient seed, are being tested in the phenotyping screen against both aphid species.

- F3 generations of the very best lines from the *T. monococcum* MDR037 x MDR045 and MDR037 x MDR657 crosses will be tested against both aphid species.

- Once screened, plants will are being vernalised and will be taken to F4.

WGIN 3

Development and exploitation of *Triticum monococcum* germplasm resources

Vanessa McMillan
Kim Hammond-Kosack

Wheat Genetic Improvement Network

Department for Environment Food & Rural Affairs

WGIN MM 12th January 2017

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ROTHAMSTED RESEARCH

Exploiting *Triticum monococcum* as a novel source of genetic diversity for improvement of hexaploid wheat

T. monococcum
wheat ear

- The diploid wheat, *T. monococcum* ($A^m A^m$) was widely cultivated during early cereal farming on poorer soils

- After the Bronze Age *T. monococcum* cultivation diminished

- A^m genome not directly involved in the generation of
  - modern durum wheat (tetraploid)
  - common bread wheat (hexaploid)

Genotyping *Triticum monococcum* collection

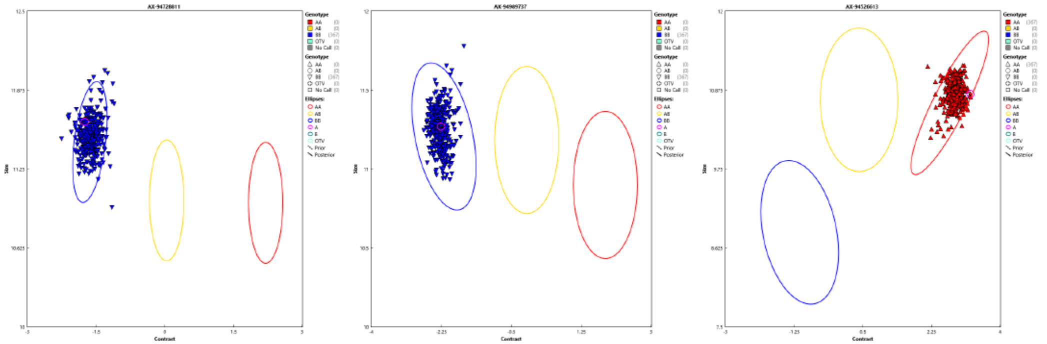
35K wheat breeders array at Bristol University

- 202 Tm genotypes from RRes collection
- MDR037 x MDR046 mapping population (79 lines, take-all resistance)
- MDR037 x MDR229 mapping population (85 lines, root strength)

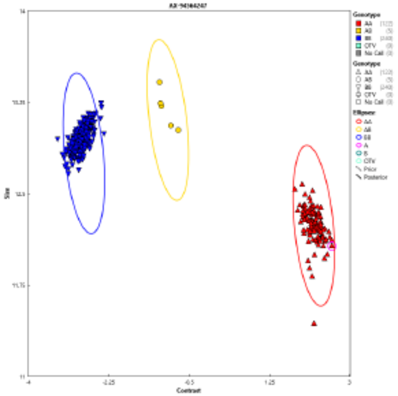
Genotyping *Triticum monococcum* collection

Majority of SNPs are monomorphic

ConversionType	Count	Percentage
MonoHighResolution	14019	39.891%
Other	11719	33.347%
NoMinorHom	3947	11.231%
OTV	3177	9.04%
PolyHighResolution	1210	3.443%
CallRateBelowThreshold	1071	3.048%



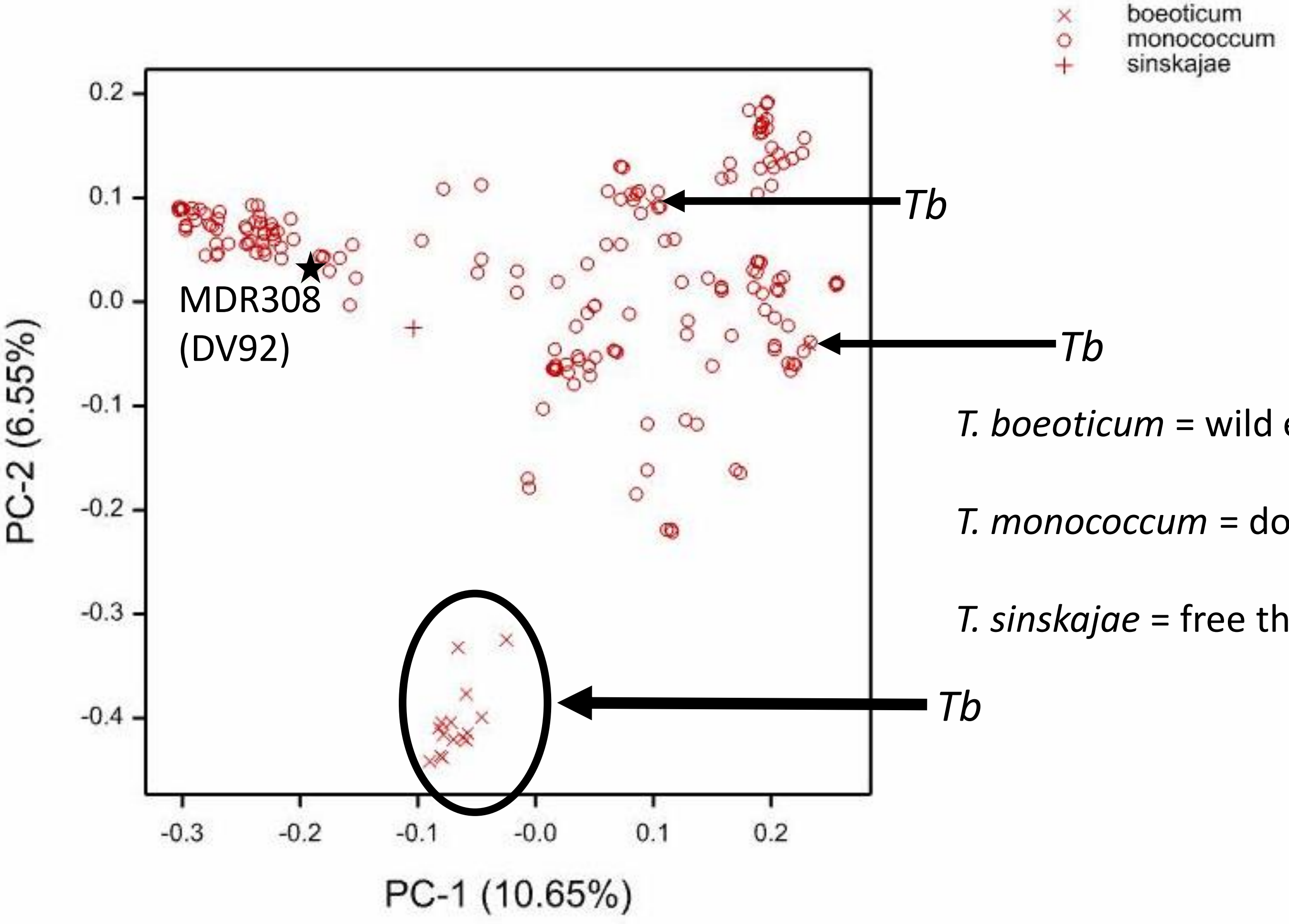
Polymorphic SNPs (after visual checking of cluster plots) = 1124 = 3.2%



Chromosomal location of SNPs in *T. aestivum*

A GENOME	38.61%
B GENOME	31.81%
D GENOME	29.58%

Triticum monococcum collection (n = 202)
Principal Coordinates Analysis



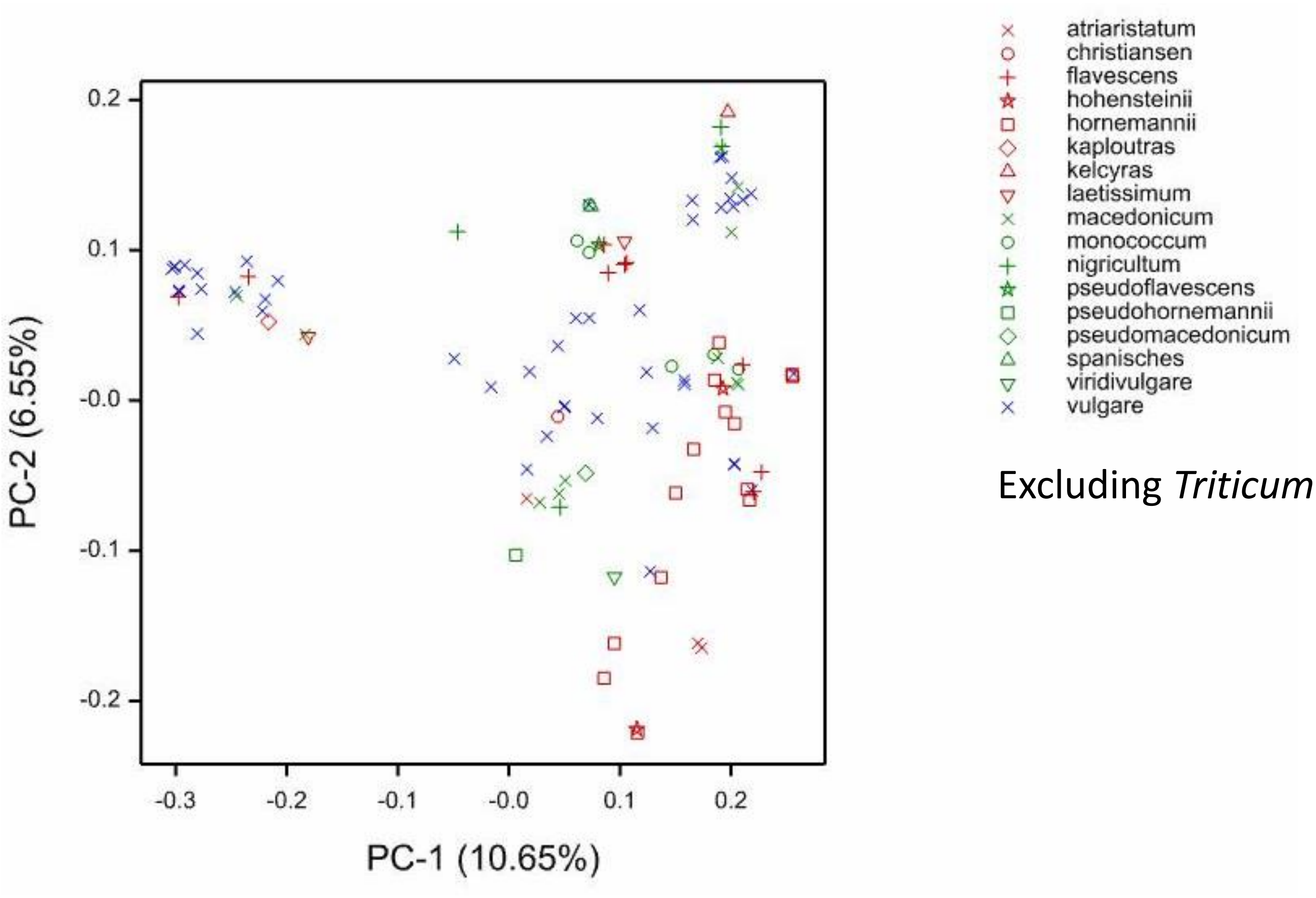
T. boeoticum = wild einkorn wheat

T. monococcum = domesticated einkorn wheat

T. sinskajae = free threshing natural mutant of *Tm*

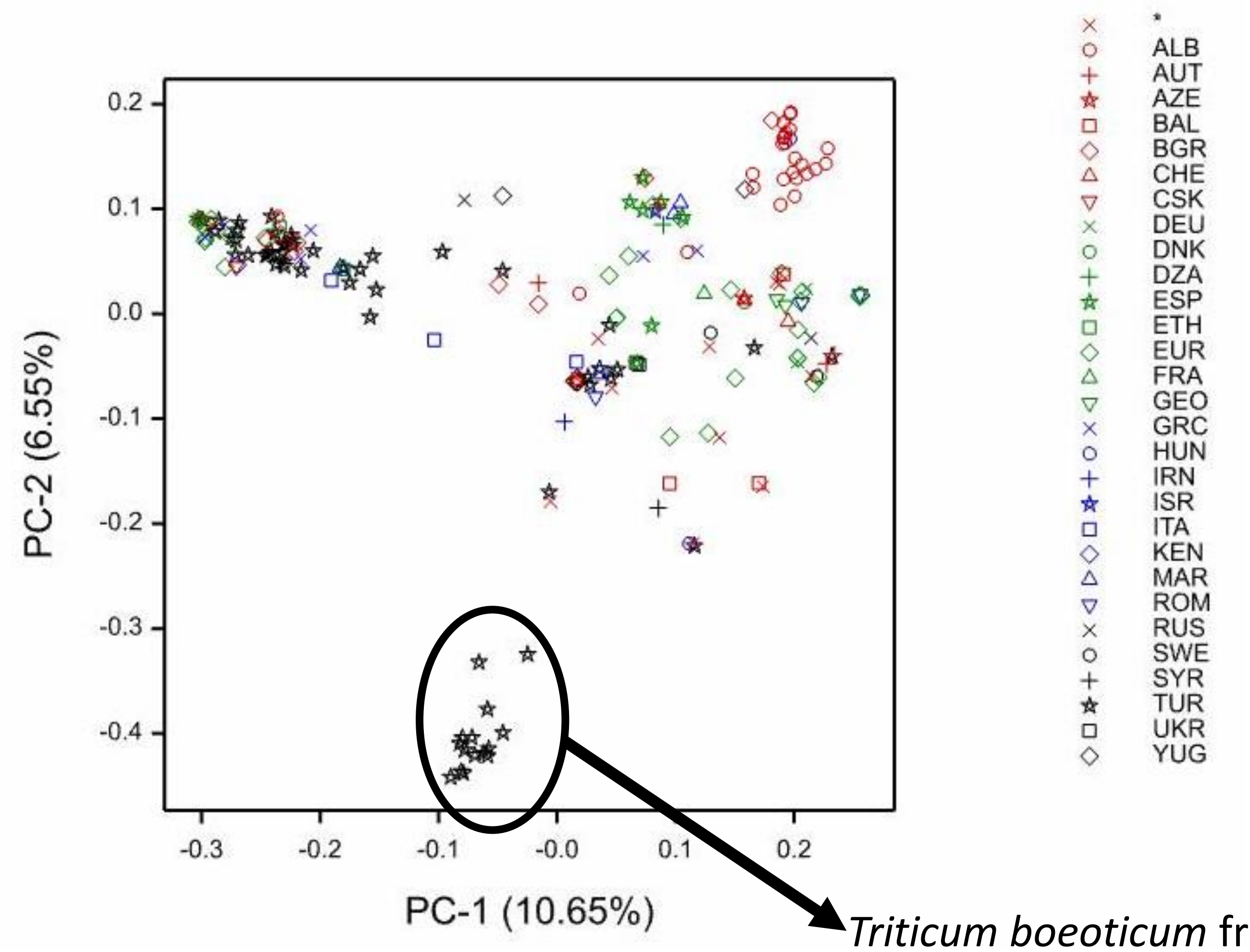
202 *Tm* genotypes, 1124 markers

Genotypic diversity is not closely associated with *Tm* variety/subspecies



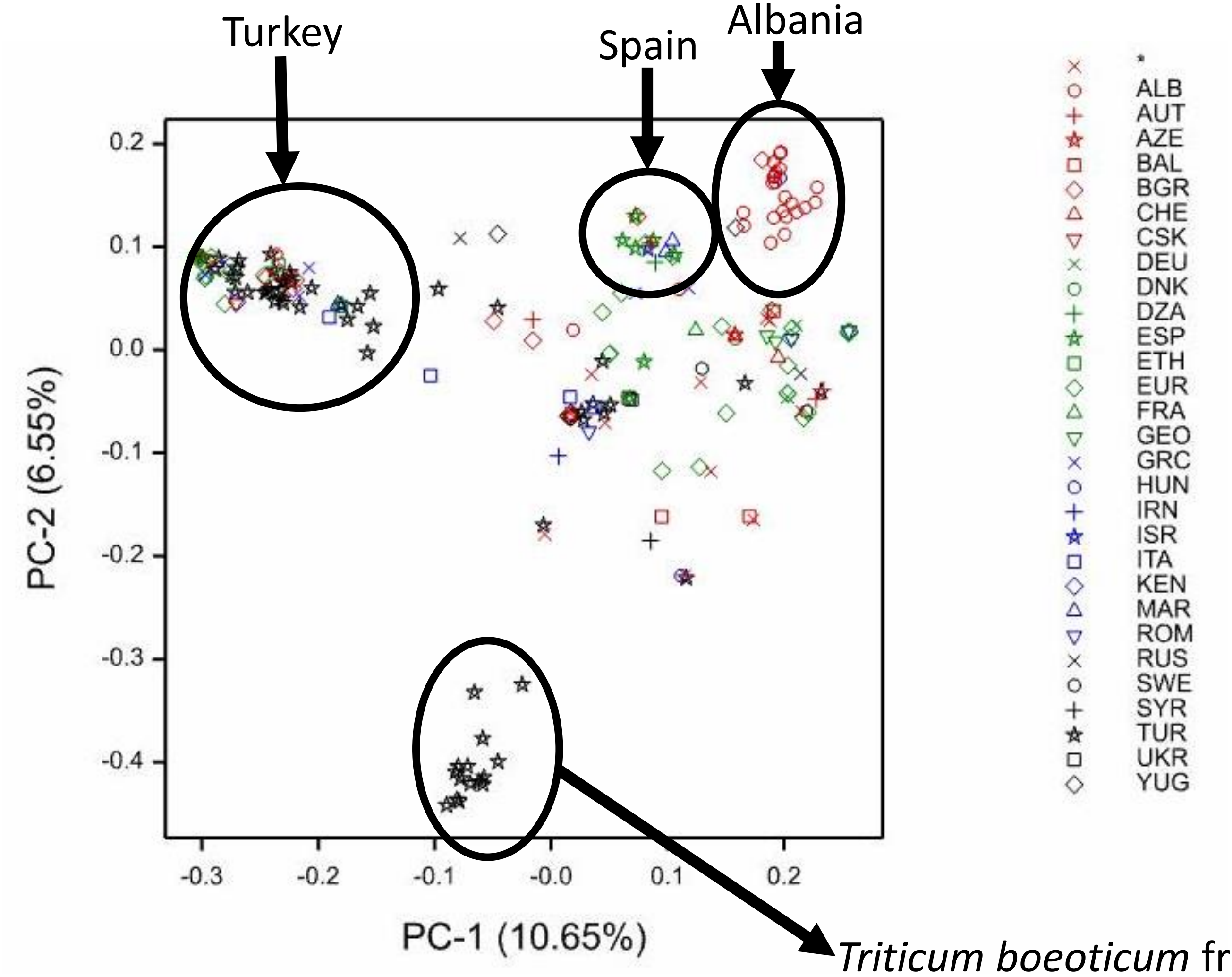
Excluding *Triticum boeoticum*

Genotypic diversity is partially associated with country of origin



Triticum boeoticum from Turkey

Genotypic diversity is partially associated with country of origin



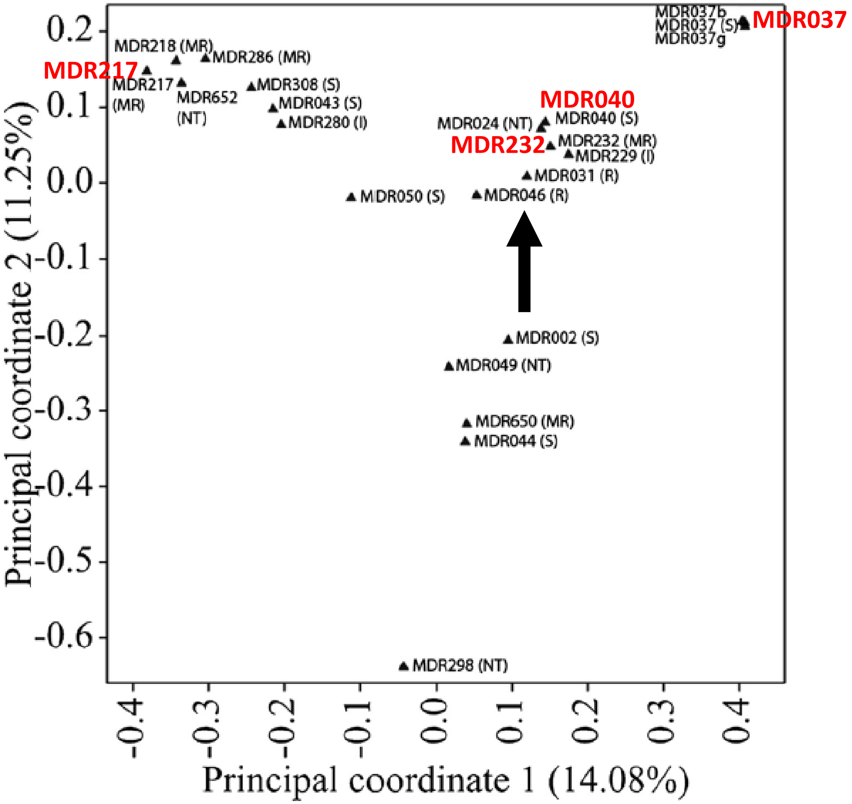
Turkey

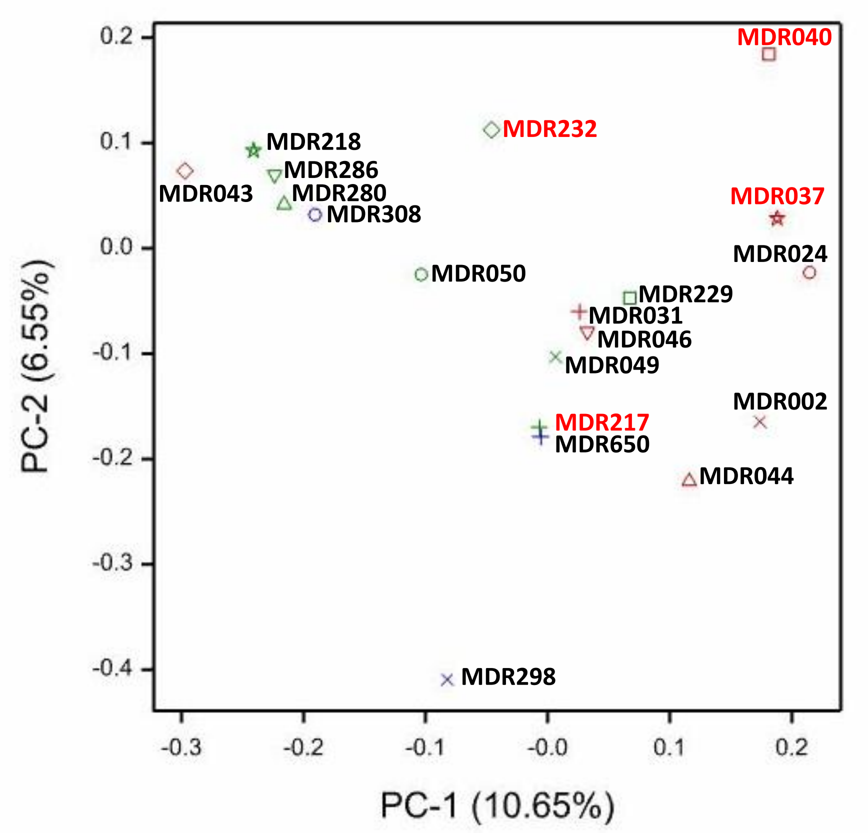
Spain

Albania

Triticum boeoticum from Turkey

Comparison with DArT marker diversity


DArT

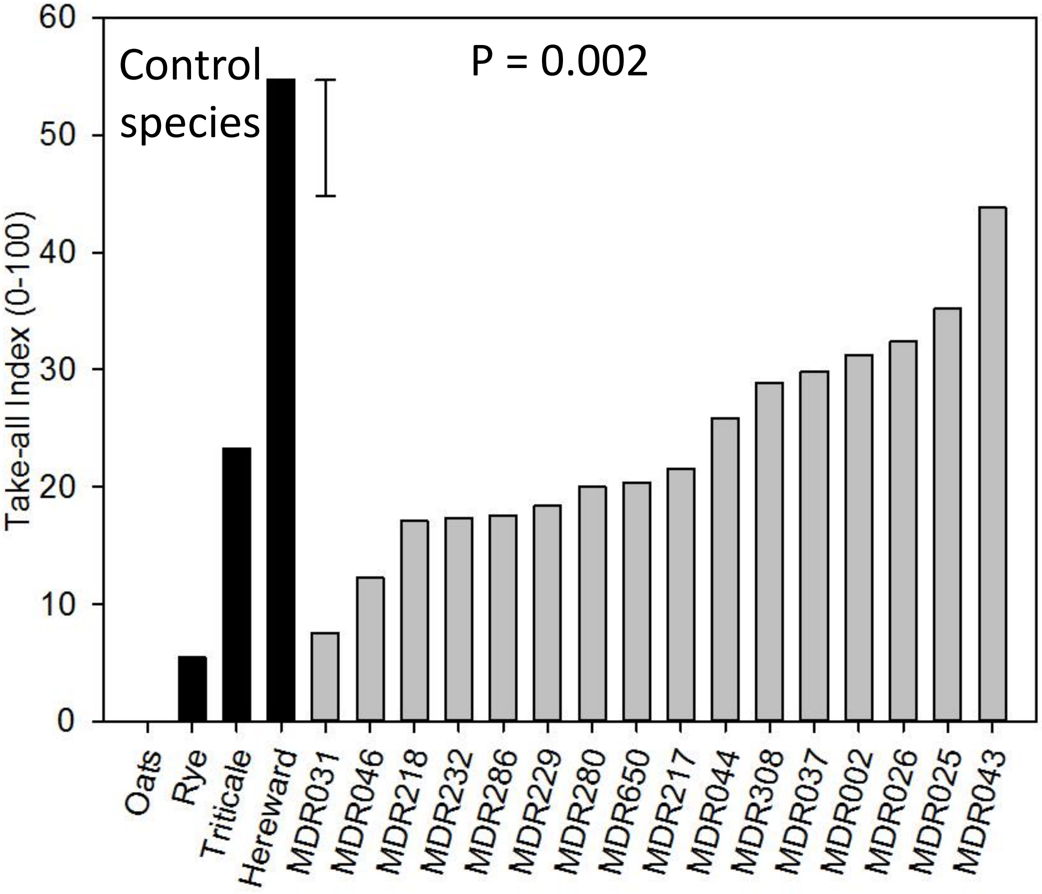

Wheat Breeder Array SNPs

20 *Tm* accessions genotyped with 1041 DArT markers in 2011

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

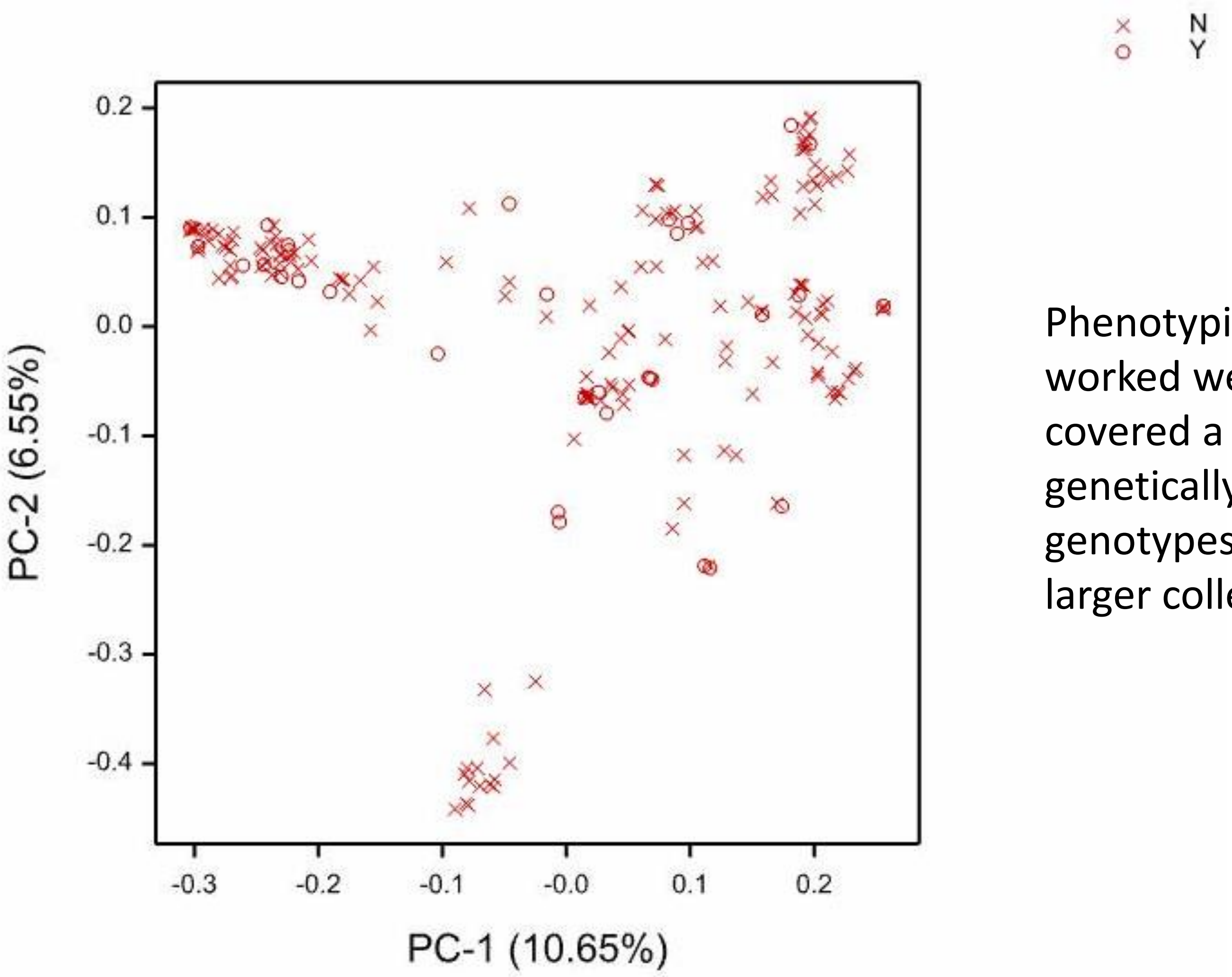
Previous selection of Tm genotypes for take-all phenotyping

- 3rd wheat field trials 2006-2011 (WGIN 1 and 2)
- **34 *T. monococcum* genotypes (AA diploid genome)** tested over 5 years
- Originally chosen to cover range of countries of origin, subspecies, growth habit



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

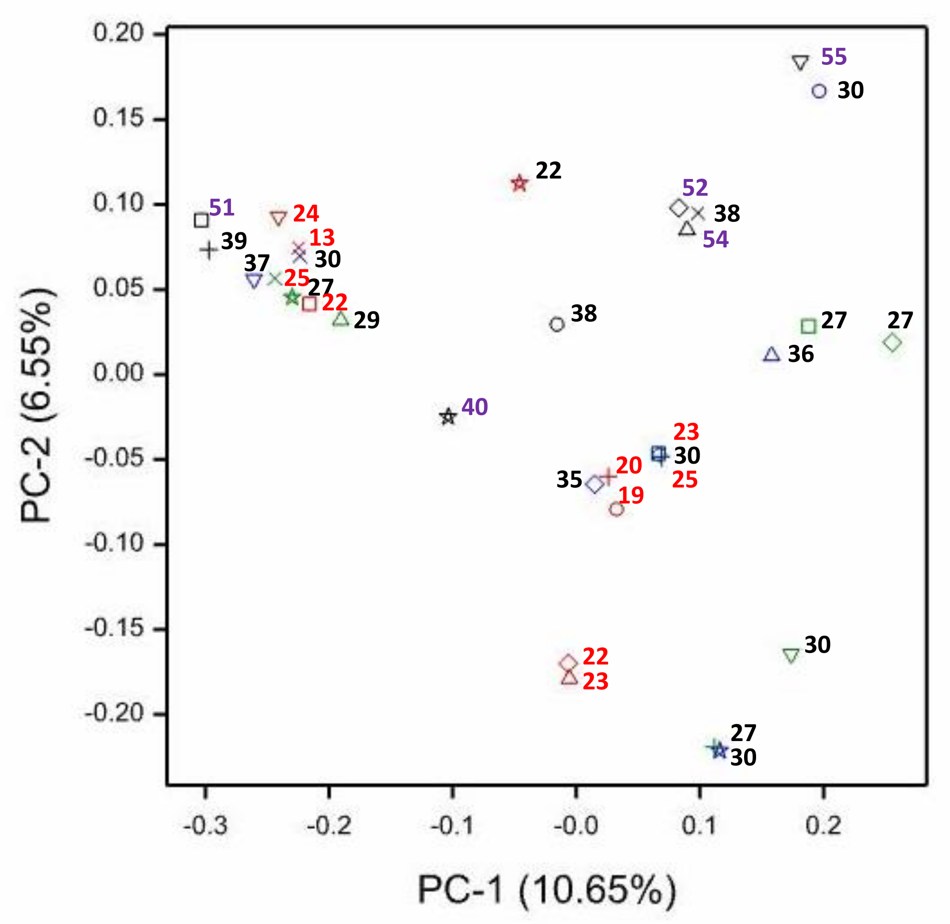
Take-all phenotyping strategy



Phenotyping strategy worked well – we have covered a range of genetically dissimilar genotypes from within larger collection

Genotypic diversity not strongly associated with take-all susceptibility

Take-all Index – combined analysis field trial data 2006, 2008-2011



Summary and next steps

- Preliminary evidence that Tm genetic diversity partially associated with country of origin

- Population structure now to be investigated in more detail using STRUCTURE software

- Aim to carry out association analyses with phenotyping data

- Yellow rust
- Take-all root disease
- Eyespot
- Septoria (Kostya Kanyuka)
- Aphid resistance (Lesley Smart and Gia Aradottir)
- Root penetration (Yaoxiang Ge and Richard Whalley)

Triticum monococcum mapping population development

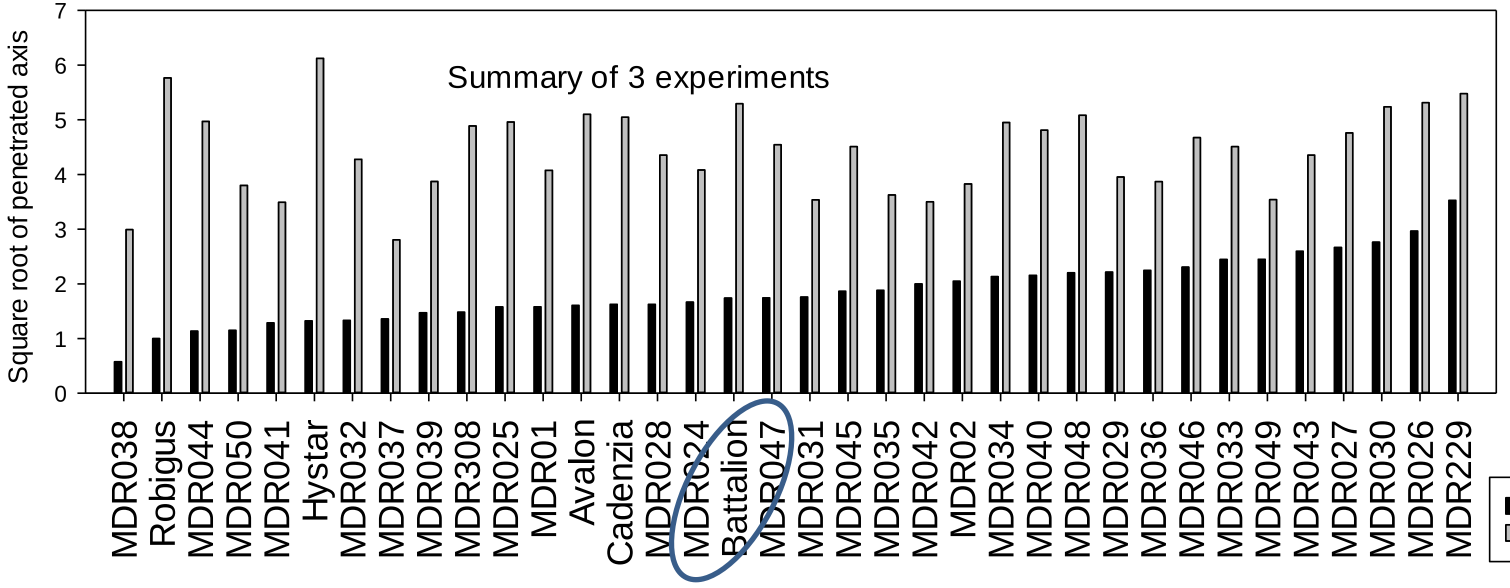
F6 mapping populations:

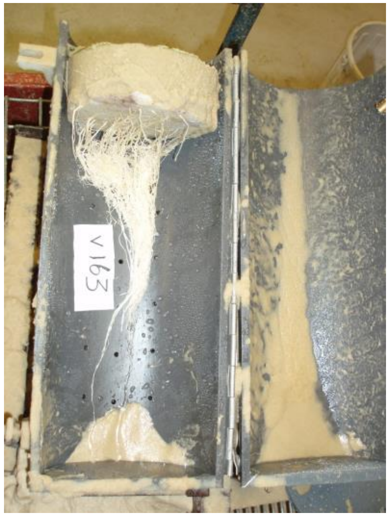
MDR037 (S) x MDR046 (R) – 79 lines (take-all resistance, 3 field seasons 2014-2016)

MDR037 x MDR229 – 85 lines (root strength, laboratory screen)

MDR031 (R) x MDR043 (S) (take-all resistance – currently being phenotyped, drilled in 3rd wheat field trial autumn 2016)

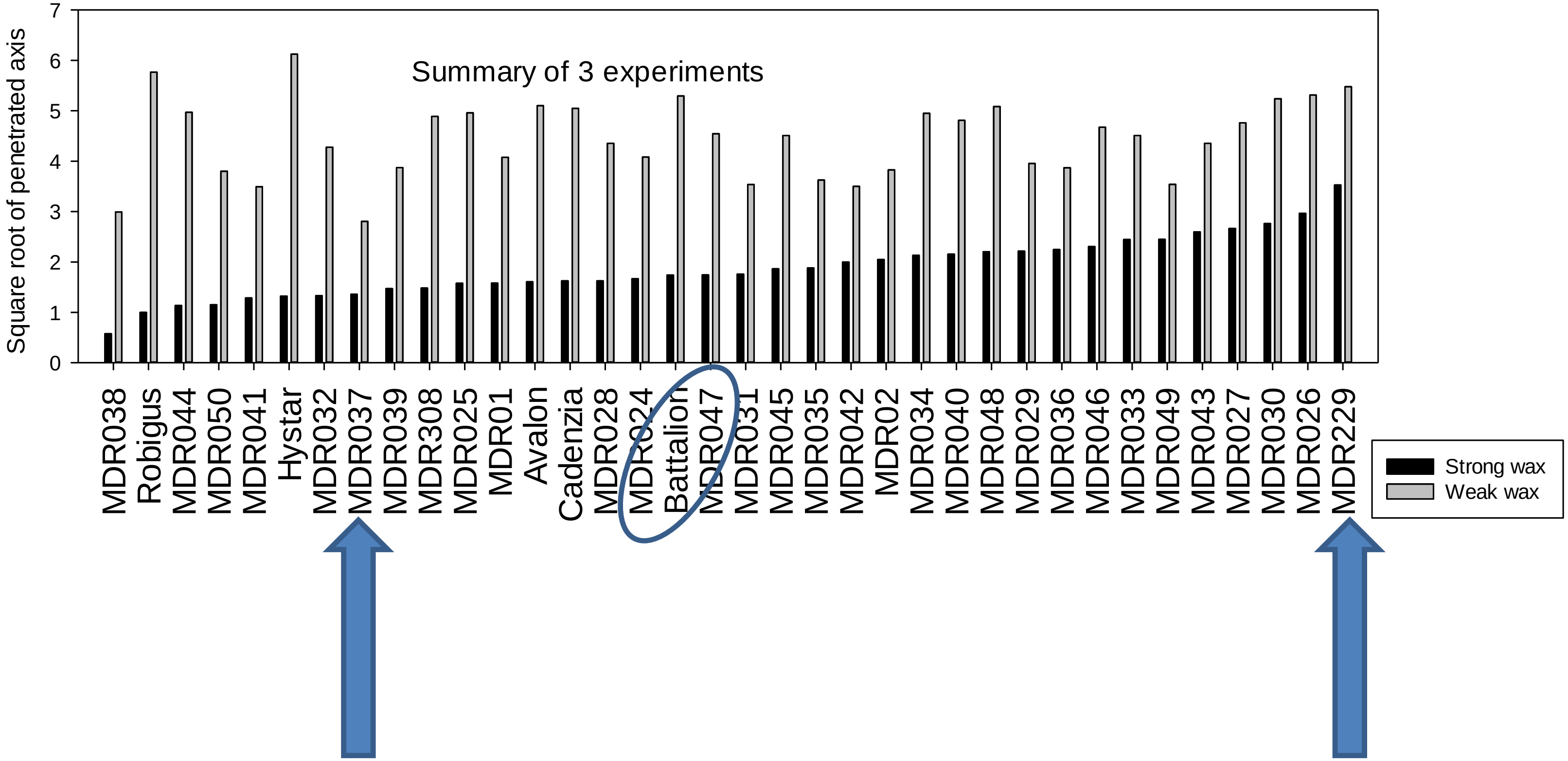
Can root penetration be improved?





Yaoxiang Ge and Richard Whalley

Can root penetration be improved?

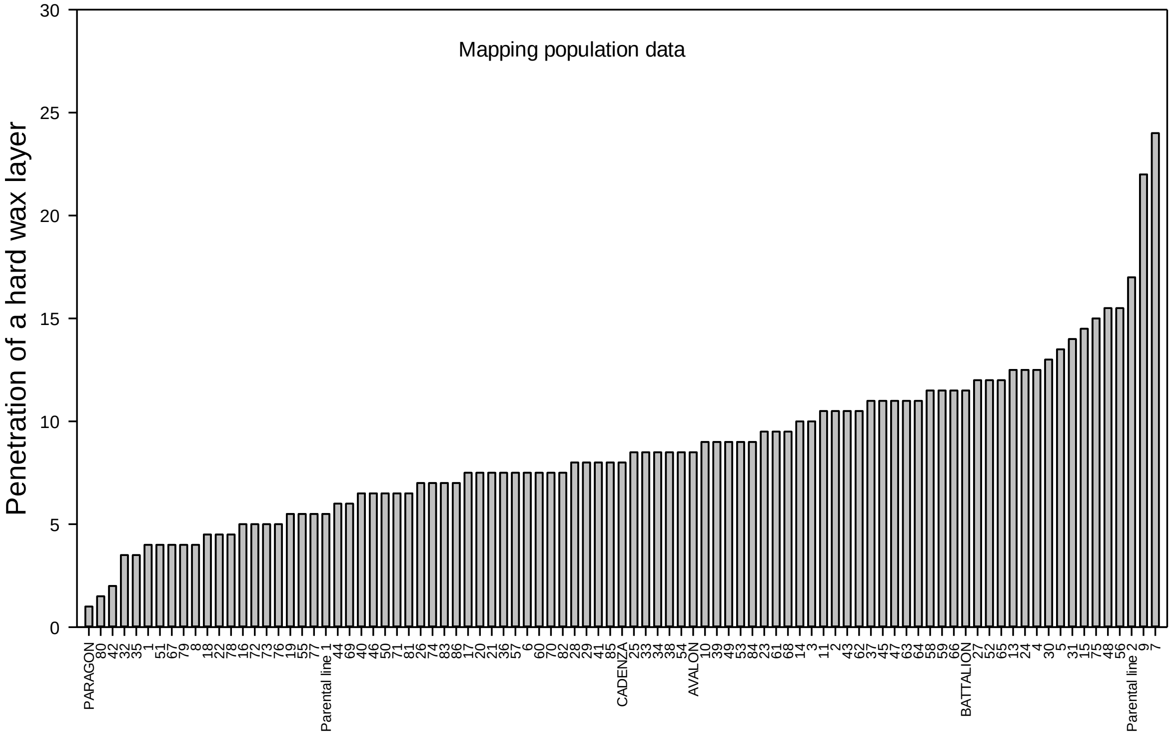


Existing mapping population

Yaoxiang Ge and Richard Whalley

Penetration of strong layers by roots

MDR037 (poor root penetration) x MDR229 (strong root penetration) mapping population

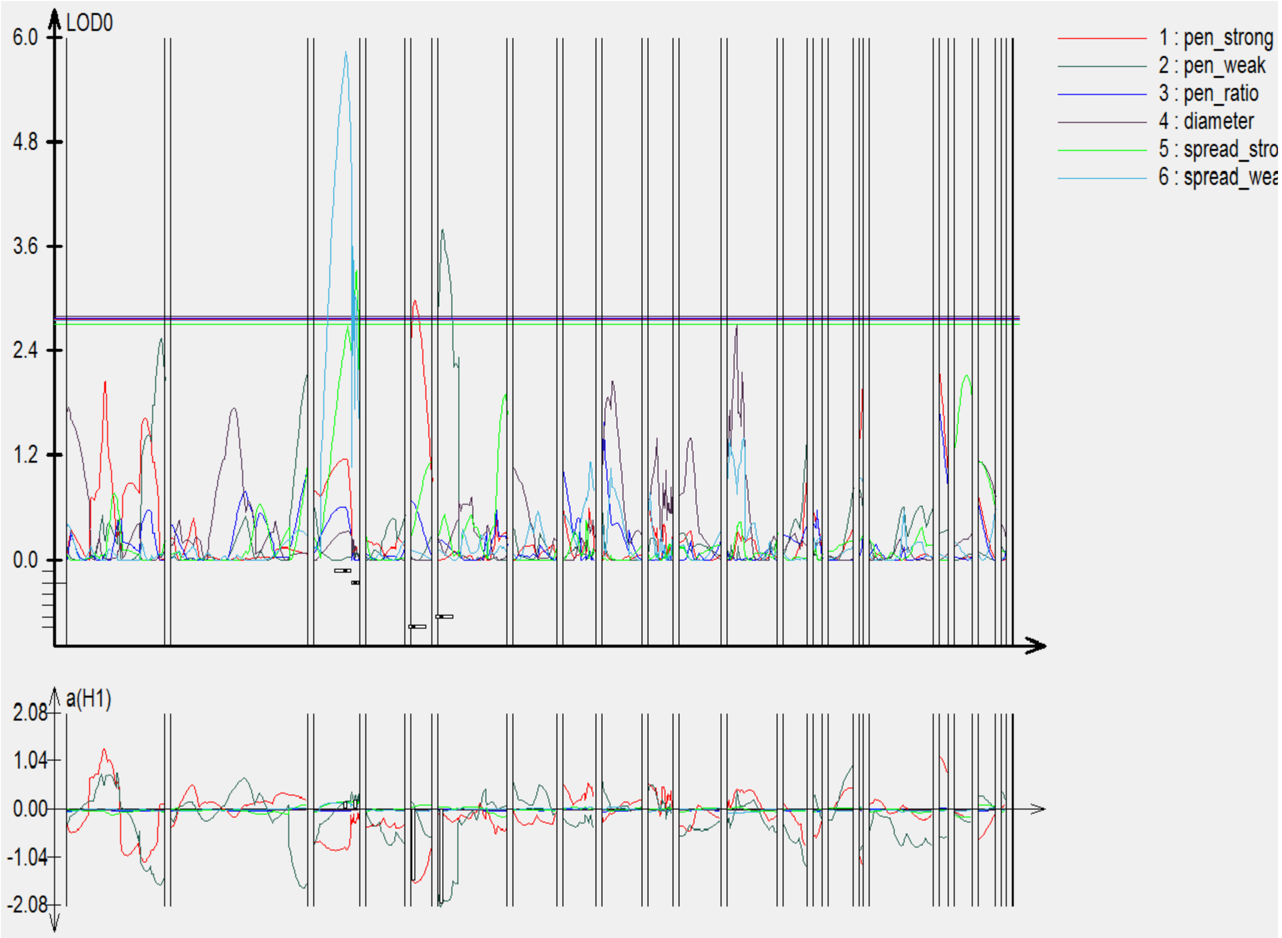


Comprehensive data set to search for QTLs including root number, root angle, root diameter and root penetration

Yaoxiang Ge and Richard Whalley

Penetration of strong layers by roots

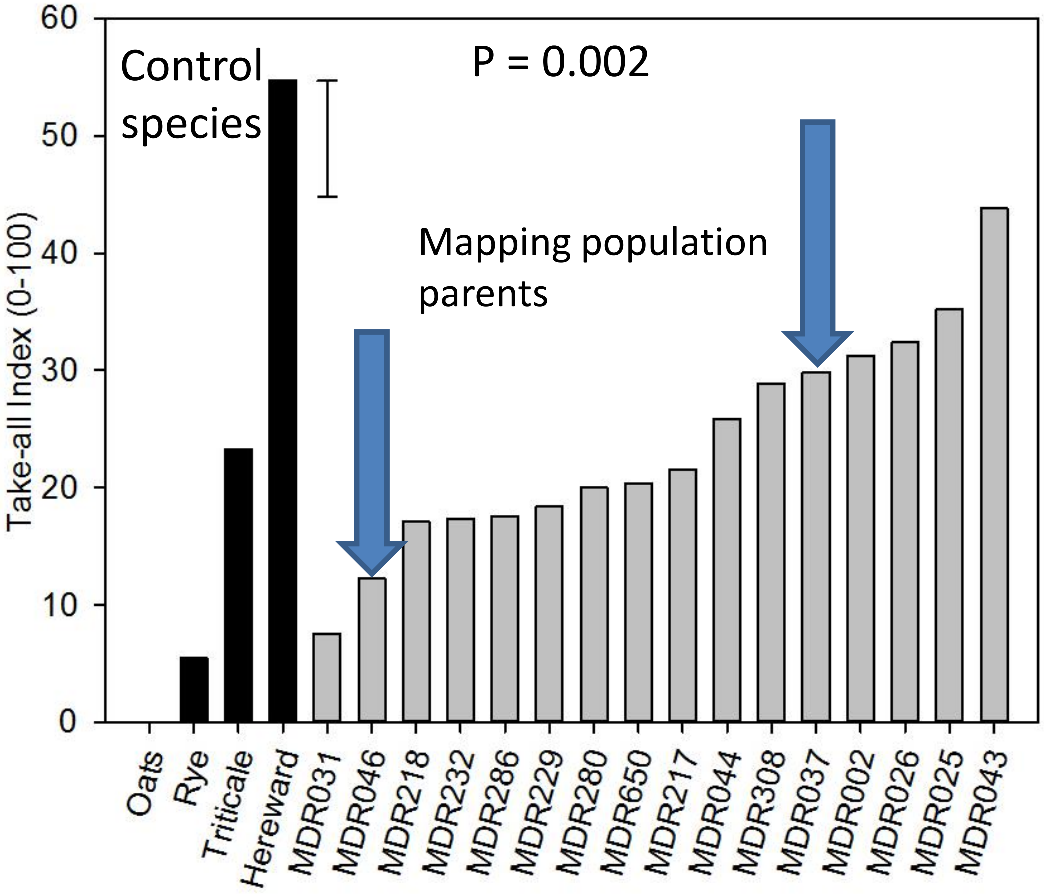
MDR037 (poor root penetration) x MDR229 (strong root penetration) mapping population



85 lines, 278 markers

Take-all mapping population development

- 3^{rd} wheat field trials 2006-2011 (WGIN 1 and 2)
- **34 *T. monococcum* genotypes (AA diploid genome)** tested over 5 years



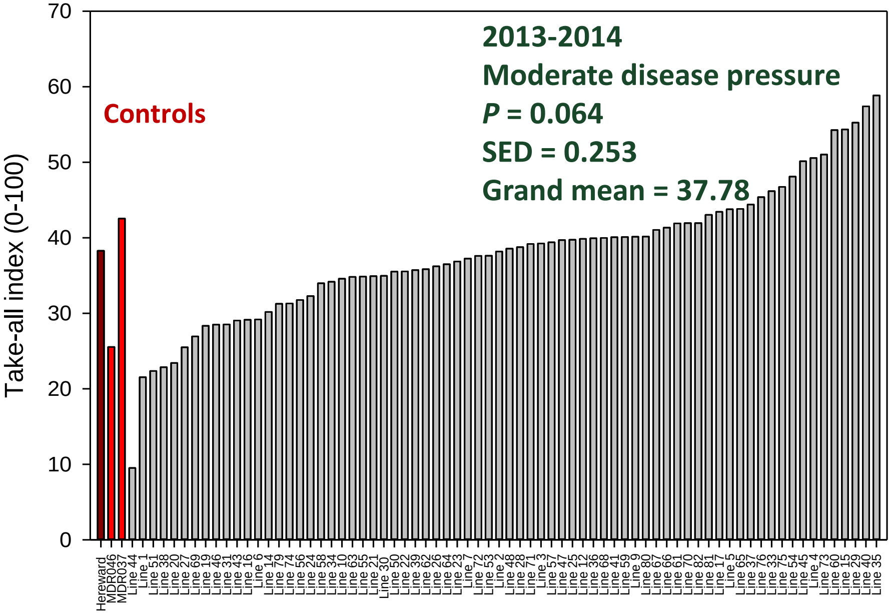
McMillan et al. (2014) BMC Plant Biology 14: 212

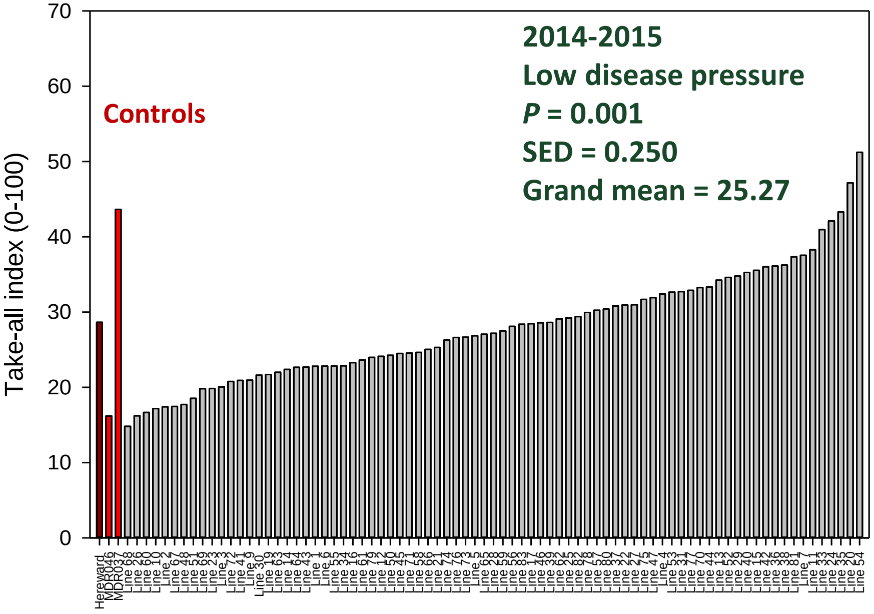
Take-all root resistance
MDR037 (S) X MDR046 (R)

3rd wheat field trials 2014, 2015 and 2016

- Randomised block design (2-5 replicates/genotype)
- Plant samples taken at GS 75 for take-all assessments

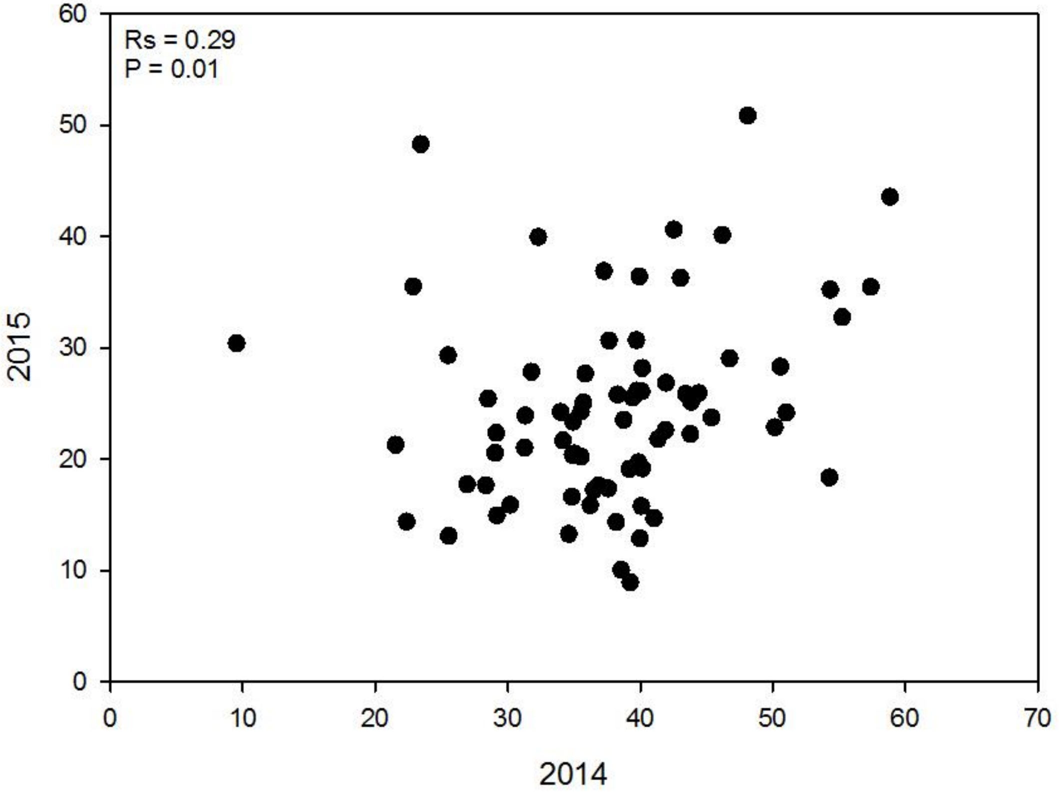
Severe winter flooding 2013-2014





PhD student Sarah-Jane Osborne

Take-all root resistance
MDR037 (S) X MDR046 (R)



Weak but significant correlation between the two field trials

PhD student Sarah-Jane Osborne

Take-all root resistance
MDR037 (S) X MDR046 (R)

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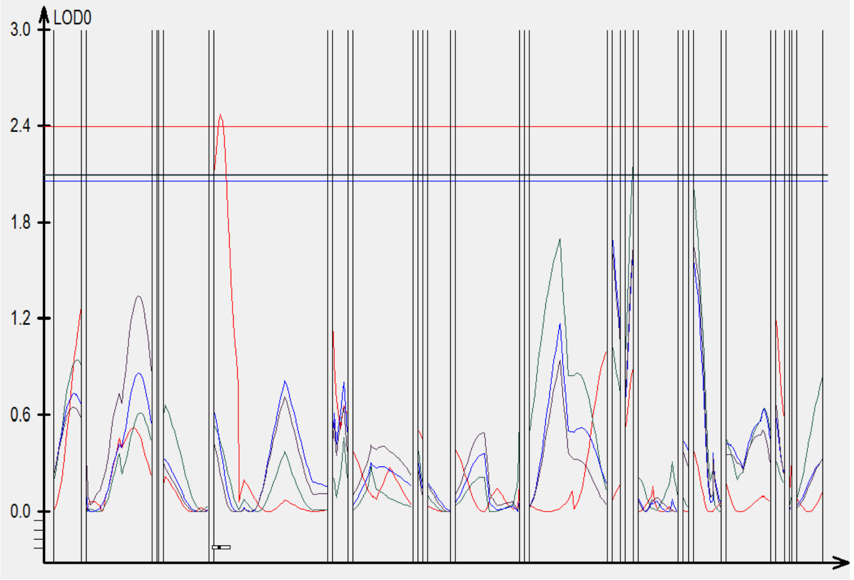
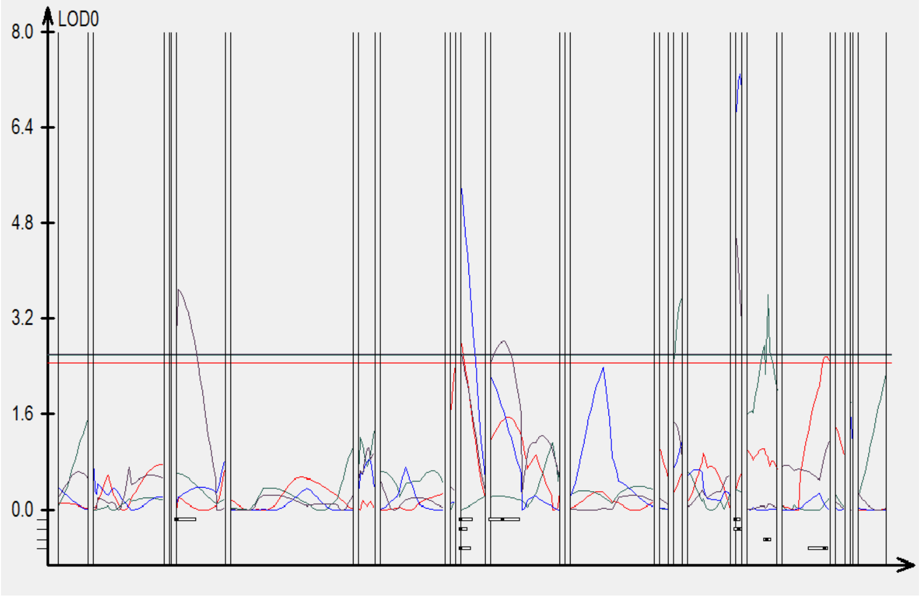
2014



2015



79 lines, 132 markers

Take-all QTLs identified on different linkage groups between the two years

🟥 1 : TAI
🟩 2 : Plant_heigh
🟦 3 : Days_anthe
🟪 4 : Days_sene

PhD student Sarah-Jane Osborne

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Summary and next steps

- MDR037 (poor penetration) x MDR229 (strong penetration) – root strength – preliminary analyses reveal multiple QTLs
- MDR037 (take-all susceptible) x MDR046 (take-all resistant) – QTLs different between field seasons – perhaps exacerbated by flooding of 2013-2014 field trial
- SSR genotyping to be carried out to improve linkage maps
- MDR031 (take-all resistant) x MDR043 (take-all susceptible) F_6 take-all phenotyping trial drilled autumn 2016, population still to be genotyped
- Strategies for introgression into hexaploid wheat being tested – using durum wheat as a bridging species

Many thanks to

Gail Canning
Joseph Moughan
Sarah-Jane Osborne
Richard Gutteridge
Kim Hammond-Kosack

Undergraduate summer students
Erin Baggs
Eleanor Leane
Tessa Reid
Laurie Neal
Alex Chambers-Ostler
Leanne Freeman

Mike-Hammond-Kosack – crossing

Rodger White - statistics

RRes farm and glasshouse staff

Soil Physics Group
Yaoxiang Ge
Colin Webster
Rhys Ashton
Richard Whalley

Tm introgression into durum and hexaploid wheat

T. monococcum source genotypes

MDR 308 (DV92) **- *TmStb1* locus mediated resistance to *Zymoseptoria tritici***

MDR 031 - Seedling and adult plant root resistance to the take-all fungus

(*Gae*

MDR 049 - Seedling and adult plant resistance to two aphid species

o Bird cherry-oat aphid *R*

o Grain aphid *Sitobion av*

Towards introgressing *TmStb1* into bread wheat

Current strategy (so far not very successful)

cross *T. monococcum* (*Tm*) to *T. aestivum* cv. Paragon *ph-1*
embryo rescue in F_1
backcross the hybrid to *T. aestivum* cv. Paragon

Trying two alternative strategies

Strategy #1

colchicin treat *Tm* to produce autotetraploid
cross *Tm* autotetraploid directly to wild-type hexaploid and durum wheat

Strategy #2

use tetraploid durum wheat as a bridging species
cross *Tm* with durum wheat
backcross this hybrid to hexaploid wheat

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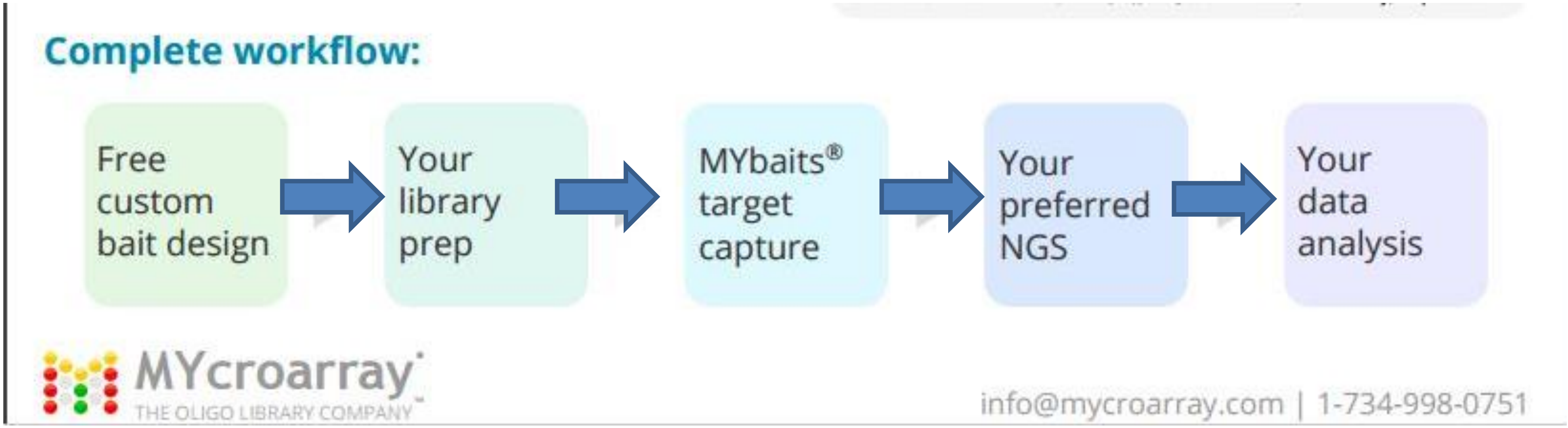
A sub-contractor project of WGIN 3

Michael Hammond-Kosack (WGIN PA)

Update for WGIN3 MM @ KWS
January 12th 2017



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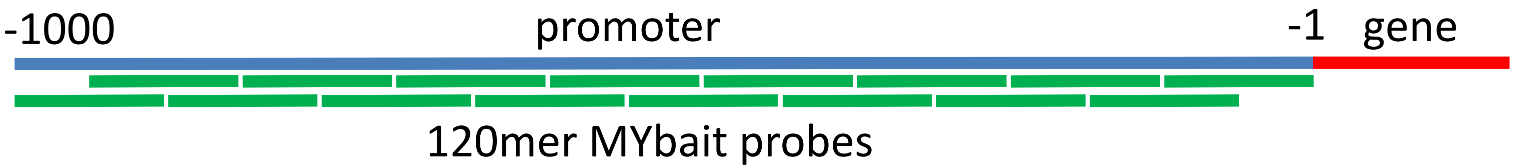
Funding & Design

- Defra’s WGIN 3 funding for this sub-contractor project allowed for the use of **20,000 MYbaits probes** and **96 “Reactions”** (which equates to **96 Wheat Cultivars genomic DNA**)

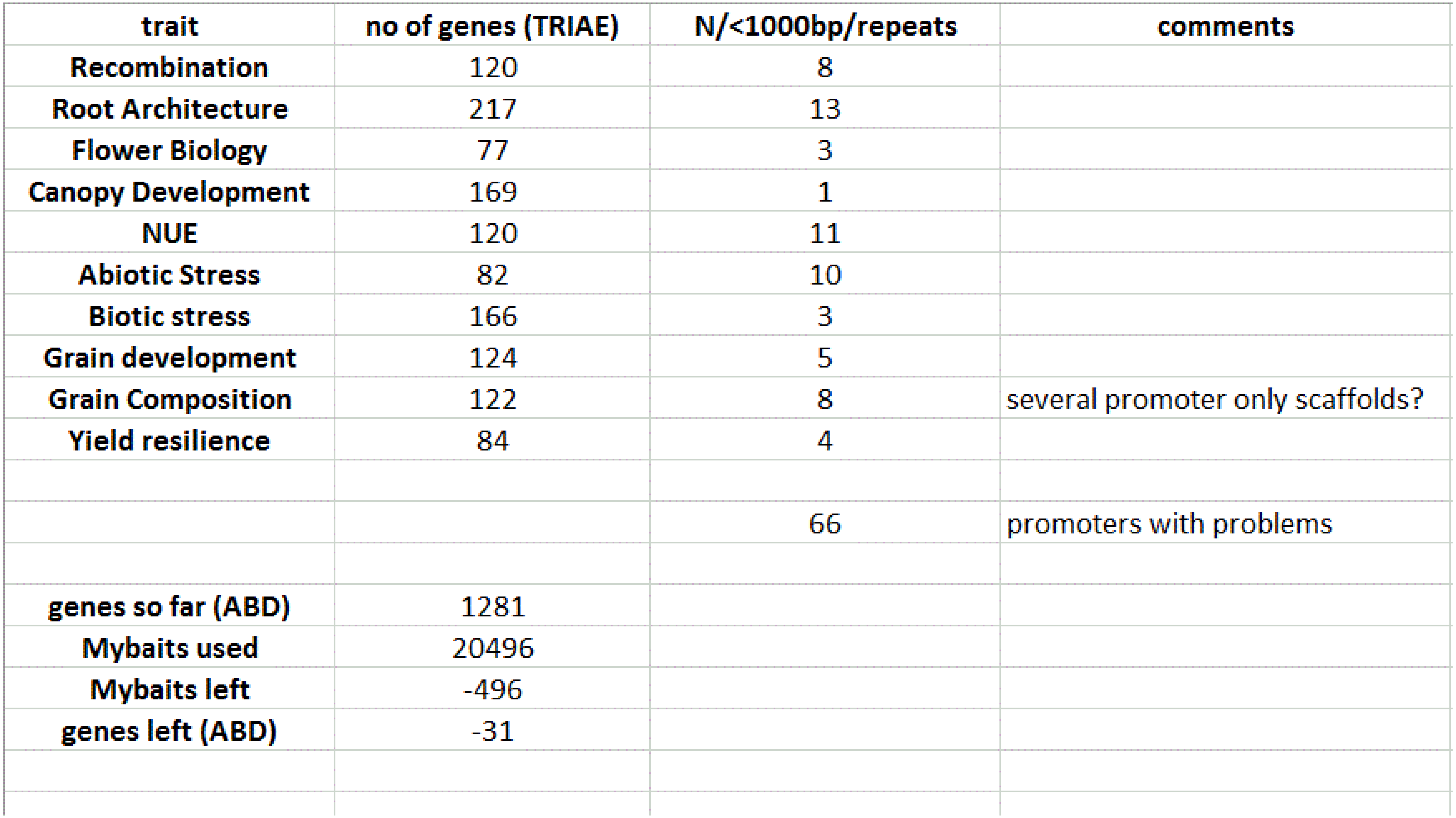
- Every **1000bp of promoter** sequence/gene requires **16 overlapping probes** for twofold coverage:



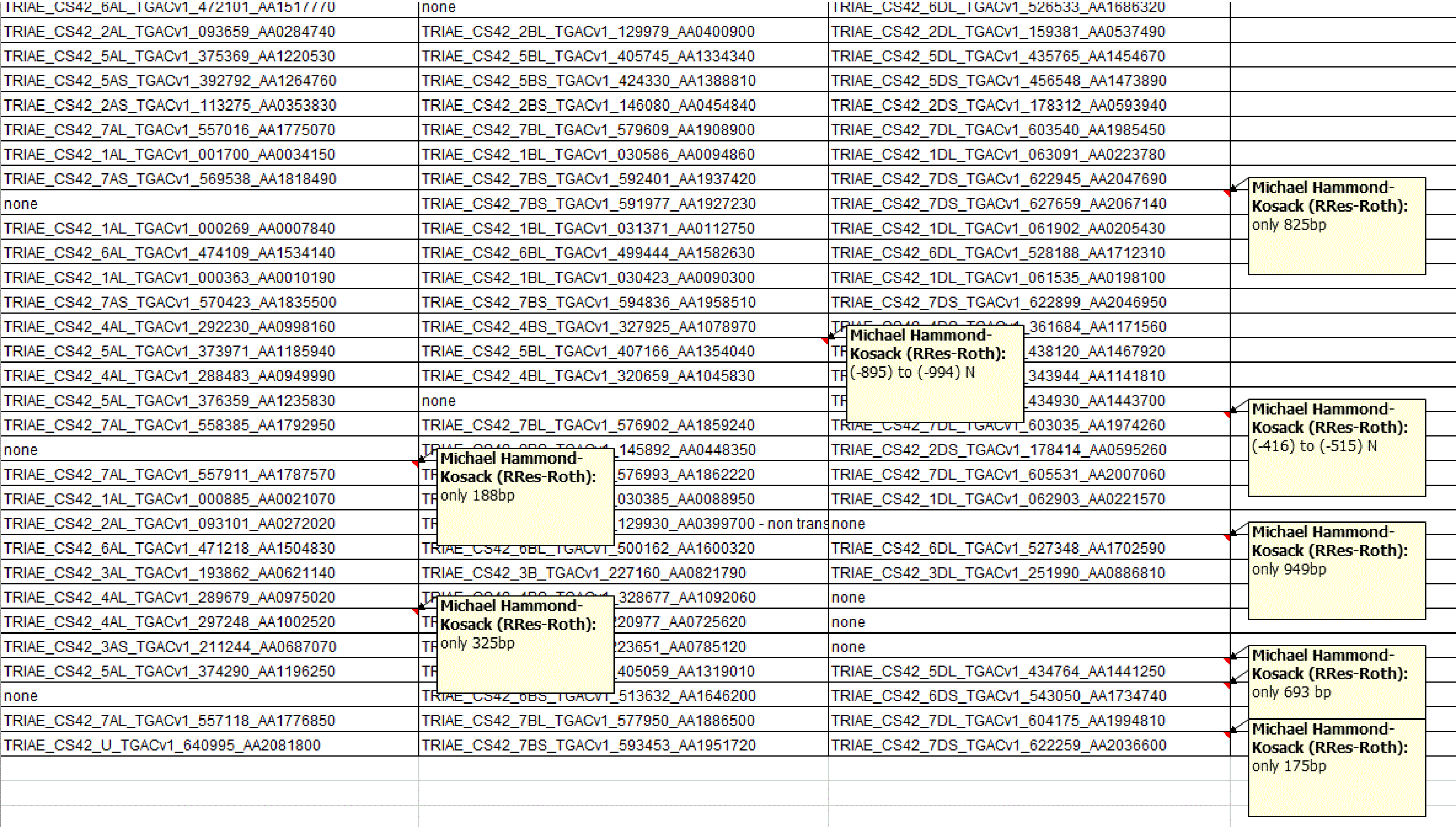
- 3 wheat homoeologues (A, B, D) **48 Mybait probes** / gene.

- Thus Defra’s funding will allow for the capture of **416 promoter sequences**.

Summary of collected genes



trait	no of genes (TRIAE)	N/<1000bp/repeats	comments
Recombination	120	8	
Root Architecture	217	13	
Flower Biology	77	3	
Canopy Development	169	1	
NUE	120	11	
Abiotic Stress	82	10	
Biotic stress	166	3	
Grain development	124	5	
Grain Composition	122	8	several promoter only scaffolds?
Yield resilience	84	4	
		66	promoters with problems
genes so far (ABD)	1281		
Mybaits used	20496		
Mybaits left	-496		
genes left (ABD)	-31		



TRIAE_CS42_6AL_TGACv1_472101_AA1517770	none	TRIAE_CS42_6DL_TGACv1_526533_AA1686320
TRIAE_CS42_2AL_TGACv1_093659_AA0284740	TRIAE_CS42_2BL_TGACv1_129979_AA0400900	TRIAE_CS42_2DL_TGACv1_159381_AA0537490
TRIAE_CS42_5AL_TGACv1_375369_AA1220530	TRIAE_CS42_5BL_TGACv1_405745_AA1334340	TRIAE_CS42_5DL_TGACv1_435765_AA1454670
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TRIAE_CS42_7AL_TGACv1_557016_AA1775070	TRIAE_CS42_7BL_TGACv1_579609_AA1908900	TRIAE_CS42_7DL_TGACv1_603540_AA1985450
TRIAE_CS42_1AL_TGACv1_001700_AA0034150	TRIAE_CS42_1BL_TGACv1_030586_AA0094860	TRIAE_CS42_1DL_TGACv1_063091_AA0223780
TRIAE_CS42_7AS_TGACv1_569538_AA1818490	TRIAE_CS42_7BS_TGACv1_592401_AA1937420	TRIAE_CS42_7DS_TGACv1_622945_AA2047690
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TRIAE_CS42_6AL_TGACv1_474109_AA1534140	TRIAE_CS42_6BL_TGACv1_499444_AA1582630	TRIAE_CS42_6DL_TGACv1_528188_AA1712310
TRIAE_CS42_1AL_TGACv1_000363_AA0010190	TRIAE_CS42_1BL_TGACv1_030423_AA0090300	TRIAE_CS42_1DL_TGACv1_061535_AA0198100
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TRIAE_CS42_5AL_TGACv1_376359_AA1235830	none	TR 434930_AA1443700
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TRIAE_CS42_U_TGACv1_640995_AA2081800	TRIAE_CS42_7BS_TGACv1_593453_AA1951720	TRIAE_CS42_7DS_TGACv1_622259_AA2036600

Michael Hammond-Kosack (RRes-Roth):
only 825bp

Michael Hammond-Kosack (RRes-Roth):
(-895) to (-994) N

Michael Hammond-Kosack (RRes-Roth):
(-416) to (-515) N

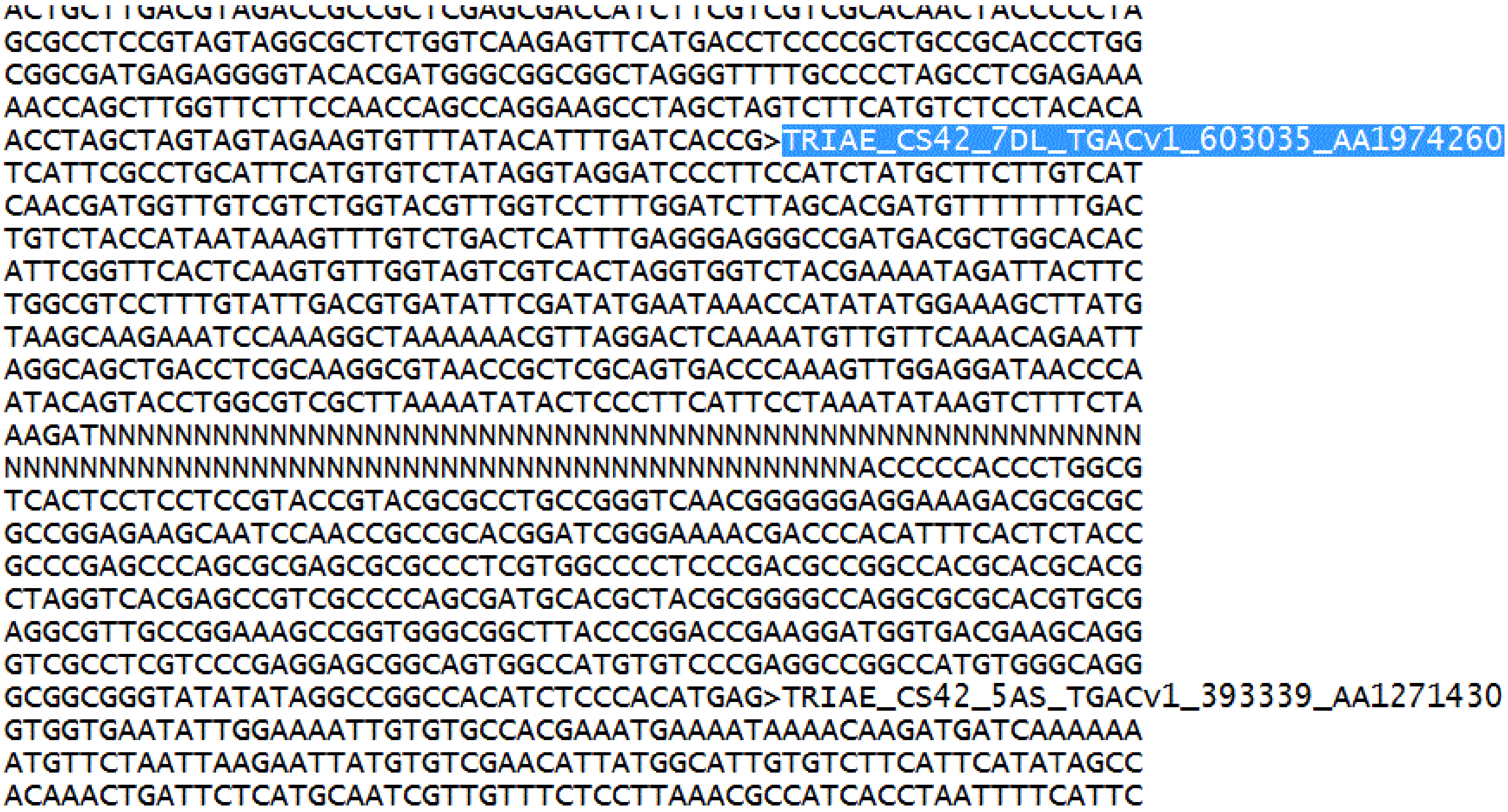
Michael Hammond-Kosack (RRes-Roth):
only 188bp

Michael Hammond-Kosack (RRes-Roth):
only 949bp

Michael Hammond-Kosack (RRes-Roth):
only 325bp

Michael Hammond-Kosack (RRes-Roth):
only 693 bp

Michael Hammond-Kosack (RRes-Roth):
only 175bp



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```

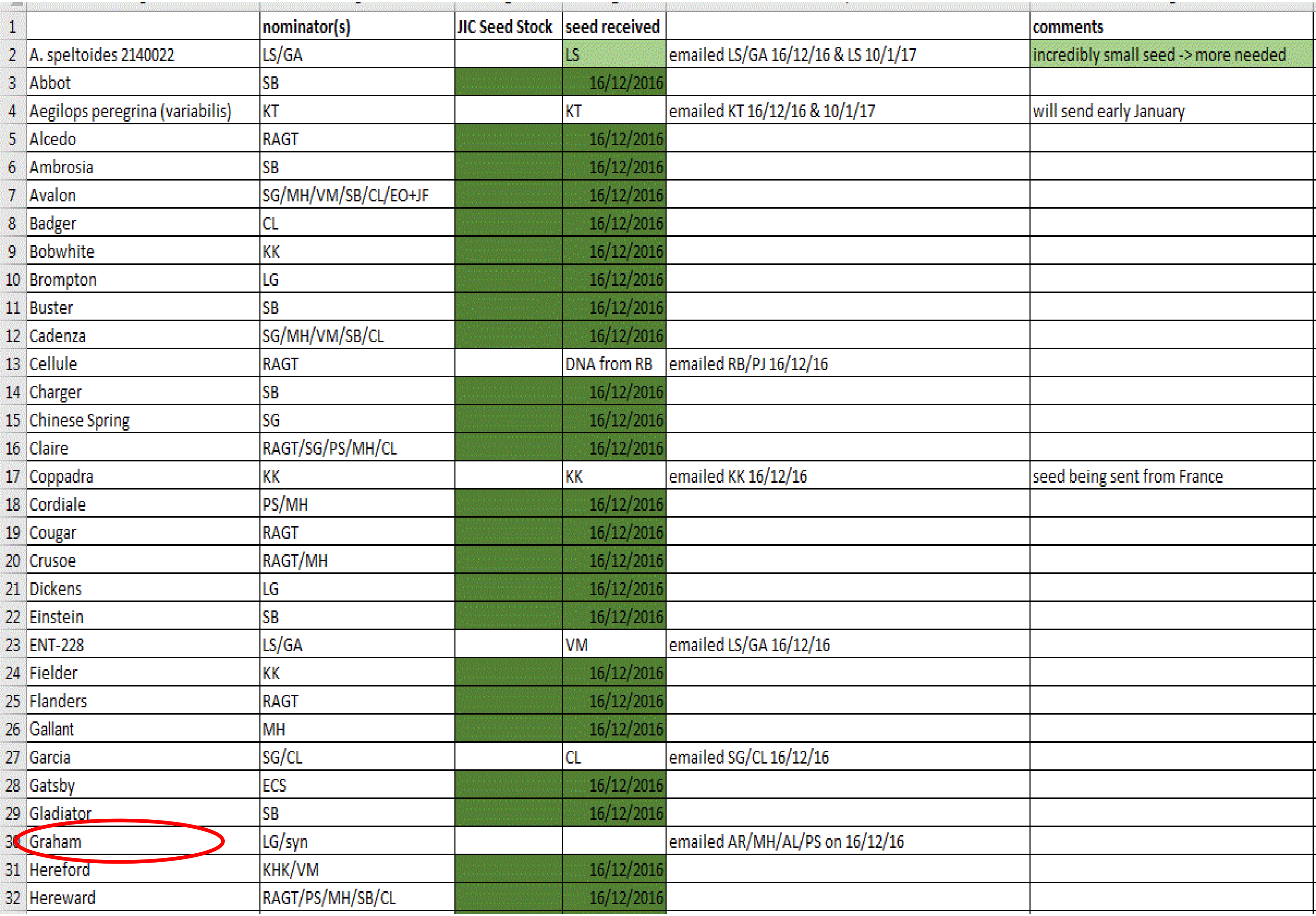
1. January 16-20: aim to **check all 66 ‘problem’ promoters** against latest
   Wheat sequences with Decypher and Genious

2. January 23-27:
   compile **ONE FASTA file with ALL sequences**

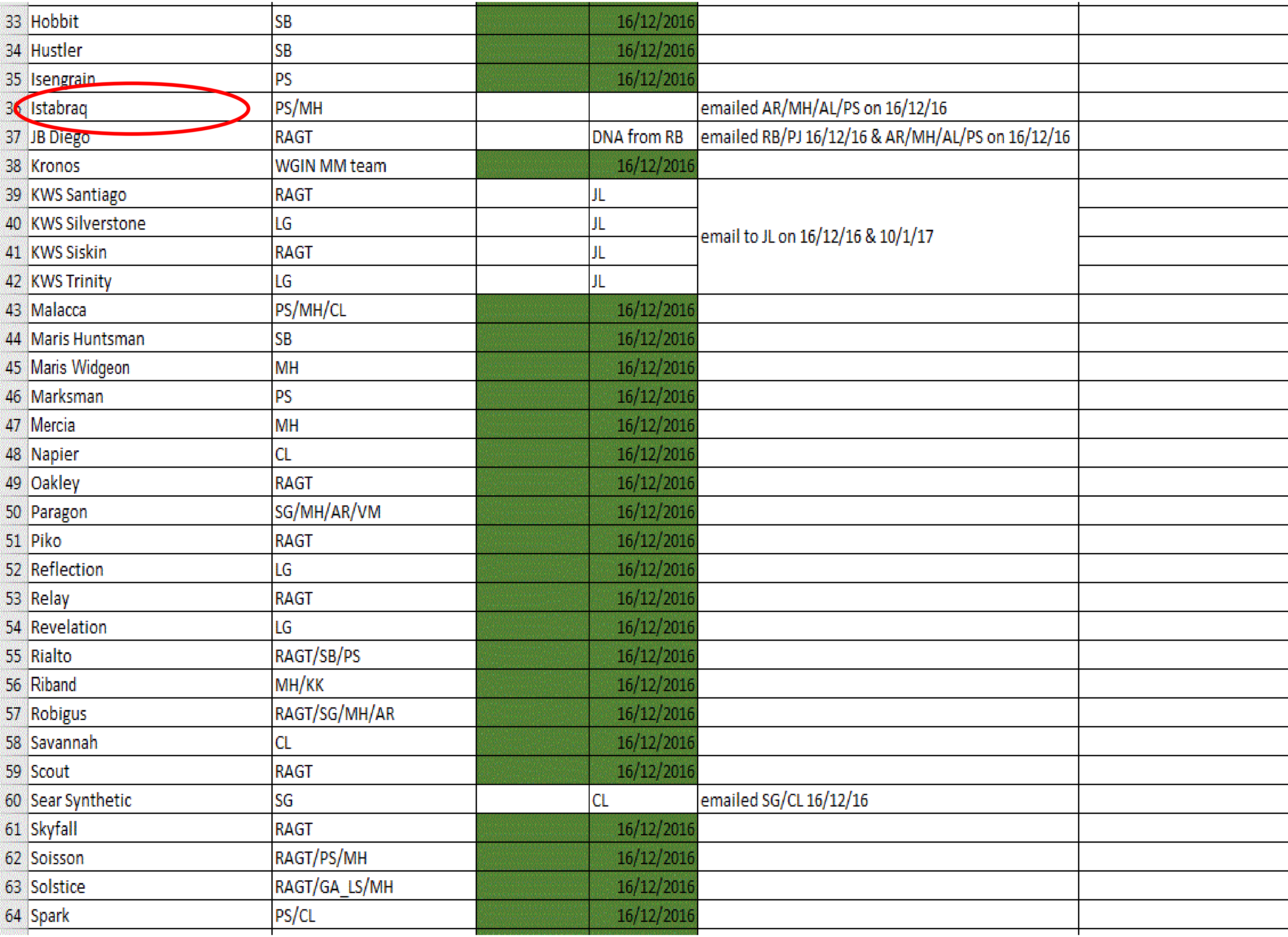
3. January 27: send **file to MYcroArray**
   approx. 6 weeks to make all 20,000 MYBaits

4. February 2017: chromosomal DNA extraction

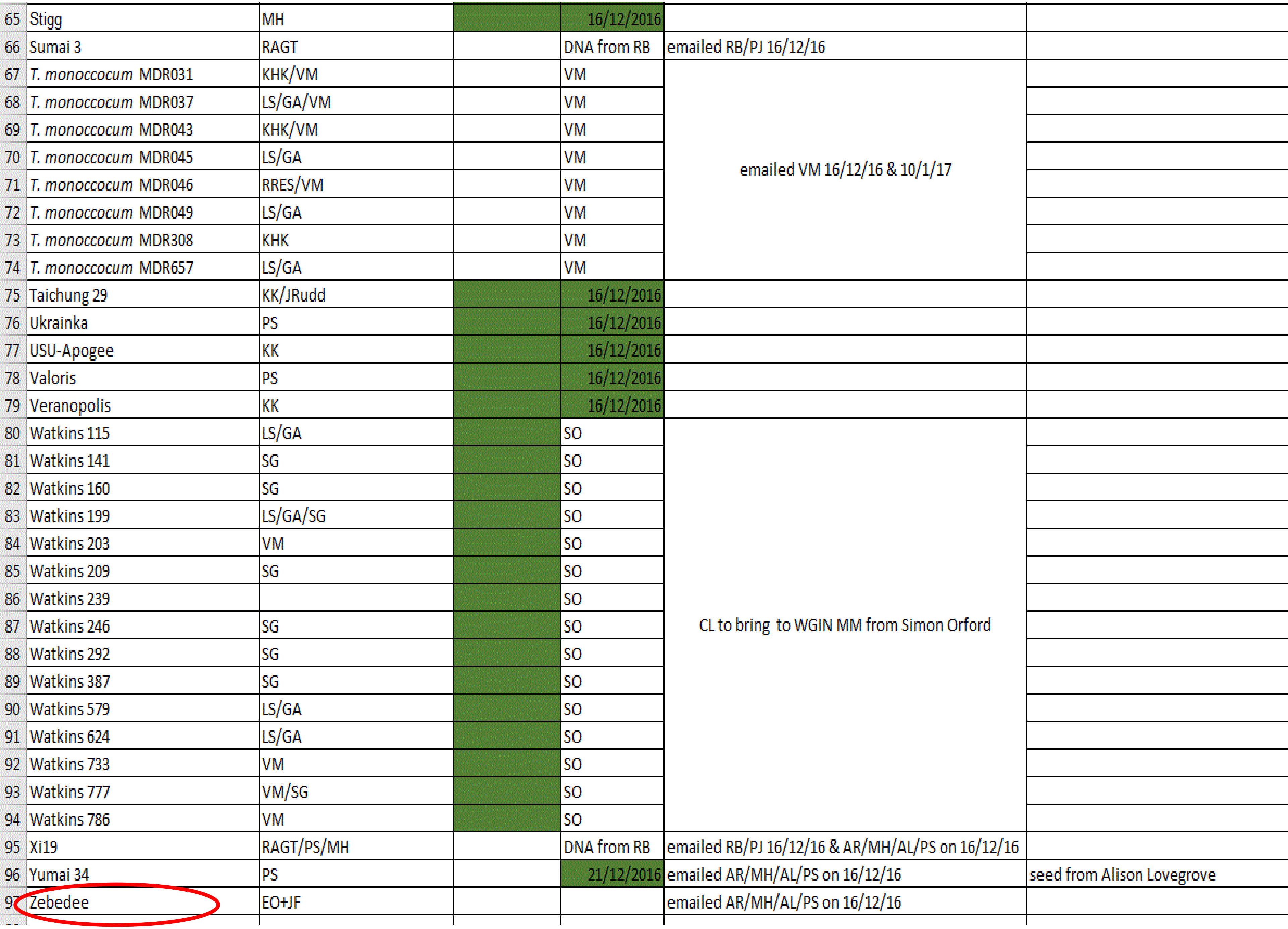
Seed collection



1		nominator(s)	JIC Seed Stock	seed received		comments
2	A. speltoides 2140022	LS/GA		LS	emailed LS/GA 16/12/16 & LS 10/1/17	incredibly small seed -> more needed
3	Abbot	SB	🟩	16/12/2016		
4	Aegilops peregrina (variabilis)	KT		KT	emailed KT 16/12/16 & 10/1/17	will send early January
5	Alcedo	RAGT	🟩	16/12/2016		
6	Ambrosia	SB	🟩	16/12/2016		
7	Avalon	SG/MH/VM/SB/CL/EO+JF	🟩	16/12/2016		
8	Badger	CL	🟩	16/12/2016		
9	Bobwhite	KK	🟩	16/12/2016		
10	Brompton	LG	🟩	16/12/2016		
11	Buster	SB	🟩	16/12/2016		
12	Cadenza	SG/MH/VM/SB/CL	🟩	16/12/2016		
13	Cellule	RAGT		DNA from RB	emailed RB/PJ 16/12/16	
14	Charger	SB	🟩	16/12/2016		
15	Chinese Spring	SG	🟩	16/12/2016		
16	Claire	RAGT/SG/PS/MH/CL	🟩	16/12/2016		
17	Coppadra	KK		KK	emailed KK 16/12/16	seed being sent from France
18	Cordiale	PS/MH	🟩	16/12/2016		
19	Cougar	RAGT	🟩	16/12/2016		
20	Crusoe	RAGT/MH	🟩	16/12/2016		
21	Dickens	LG	🟩	16/12/2016		
22	Einstein	SB	🟩	16/12/2016		
23	ENT-228	LS/GA		VM	emailed LS/GA 16/12/16	
24	Fielder	KK	🟩	16/12/2016		
25	Flanders	RAGT	🟩	16/12/2016		
26	Gallant	MH	🟩	16/12/2016		
27	Garcia	SG/CL		CL	emailed SG/CL 16/12/16	
28	Gatsby	ECS	🟩	16/12/2016		
29	Gladiator	SB	🟩	16/12/2016		
30	Graham	LG/syn			emailed AR/MH/AL/PS on 16/12/16	
31	Hereford	KHK/VM	🟩	16/12/2016		
32	Hereward	RAGT/PS/MH/SB/CL	🟩	16/12/2016		



33	Hobbit	SB	🟩	16/12/2016	
34	Hustler	SB	🟩	16/12/2016	
35	Isengrain	PS	🟩	16/12/2016	
36	Istabraq	PS/MH			emailed AR/MH/AL/PS on 16/12/16
37	JB Diego	RAGT		DNA from RB	emailed RB/PJ 16/12/16 & AR/MH/AL/PS on 16/12/16
38	Kronos	WGIN MM team	🟩	16/12/2016	
39	KWS Santiago	RAGT		JL	email to JL on 16/12/16 & 10/1/17
40	KWS Silverstone	LG		JL	
41	KWS Siskin	RAGT		JL	
42	KWS Trinity	LG		JL	
43	Malacca	PS/MH/CL	🟩	16/12/2016	
44	Maris Huntsman	SB	🟩	16/12/2016	
45	Maris Widgeon	MH	🟩	16/12/2016	
46	Marksman	PS	🟩	16/12/2016	
47	Mercia	MH	🟩	16/12/2016	
48	Napier	CL	🟩	16/12/2016	
49	Oakley	RAGT	🟩	16/12/2016	
50	Paragon	SG/MH/AR/VM	🟩	16/12/2016	
51	Piko	RAGT	🟩	16/12/2016	
52	Reflection	LG	🟩	16/12/2016	
53	Relay	RAGT	🟩	16/12/2016	
54	Revelation	LG	🟩	16/12/2016	
55	Rialto	RAGT/SB/PS	🟩	16/12/2016	
56	Riband	MH/KK	🟩	16/12/2016	
57	Robigus	RAGT/SG/MH/AR	🟩	16/12/2016	
58	Savannah	CL	🟩	16/12/2016	
59	Scout	RAGT	🟩	16/12/2016	
60	Sear Synthetic	SG		CL	emailed SG/CL 16/12/16
61	Skyfall	RAGT	🟩	16/12/2016	
62	Soisson	RAGT/PS/MH	🟩	16/12/2016	
63	Solstice	RAGT/GA_LS/MH	🟩	16/12/2016	
64	Spark	PS/CL	🟩	16/12/2016	



65	Stigg	MH	🟩	16/12/2016		
66	Sumai 3	RAGT		DNA from RB	emailed RB/PJ 16/12/16	
67	*T. monoccocum* MDR031	KHK/VM		VM	emailed VM 16/12/16 & 10/1/17	
68	*T. monoccocum* MDR037	LS/GA/VM		VM		
69	*T. monoccocum* MDR043	KHK/VM		VM		
70	*T. monoccocum* MDR045	LS/GA		VM		
71	*T. monoccocum* MDR046	RRES/VM		VM		
72	*T. monoccocum* MDR049	LS/GA		VM		
73	*T. monoccocum* MDR308	KHK		VM		
74	*T. monoccocum* MDR657	LS/GA		VM		
75	Taichung 29	KK/JRudd	🟩	16/12/2016		
76	Ukrainka	PS	🟩	16/12/2016		
77	USU-Apogee	KK	🟩	16/12/2016		
78	Valoris	PS	🟩	16/12/2016		
79	Veranopolis	KK	🟩	16/12/2016		
80	Watkins 115	LS/GA	🟩	SO	CL to bring to WGIN MM from Simon Orford	
81	Watkins 141	SG	🟩	SO		
82	Watkins 160	SG	🟩	SO		
83	Watkins 199	LS/GA/SG	🟩	SO		
84	Watkins 203	VM	🟩	SO		
85	Watkins 209	SG	🟩	SO		
86	Watkins 239		🟩	SO		
87	Watkins 246	SG	🟩	SO		
88	Watkins 292	SG	🟩	SO		
89	Watkins 387	SG	🟩	SO		
90	Watkins 579	LS/GA	🟩	SO		
91	Watkins 624	LS/GA	🟩	SO		
92	Watkins 733	VM	🟩	SO		
93	Watkins 777	VM/SG	🟩	SO		
94	Watkins 786	VM	🟩	SO		
95	Xi19	RAGT/PS/MH		DNA from RB	emailed RB/PJ 16/12/16 & AR/MH/AL/PS on 16/12/16	
96	Yumai 34	PS		21/12/2016	emailed AR/MH/AL/PS on 16/12/16	seed from Alison Lovegrove
97	Zebedee	EO+JF			emailed AR/MH/AL/PS on 16/12/16	

Timeline

1) **December 2016**: obtain remaining gene-lists
2) **December 2016**: obtain seed for all 96 cultivars
3) **January 2017**: make chromosomal DNAs
4) **January 2017**: send FASTA file to Mycroarray for them to design Mybaits
5) **February 2017**: send chromosomal DNAs to Mycroarray to conduct the Promotome Capture Experiment.
6) **March 2017**: Next Generation Sequencing
7) **April 2017**: Sequences returned and analysed at RRes
8) **Summer 2017**: Data & Analysis released to Wheat Community