Wheat
Genetic
Improvement
Network

John Innes Centre

Tools, resources, genotyping and phenotyping

Clare Lister and Simon Griffiths

6/10/2017

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WGIN3 Projects: Griffiths' Lab

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

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WGIN3 Projects: Paragon Library

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WGIN3 Projects: Paragon Library 1. and 2.

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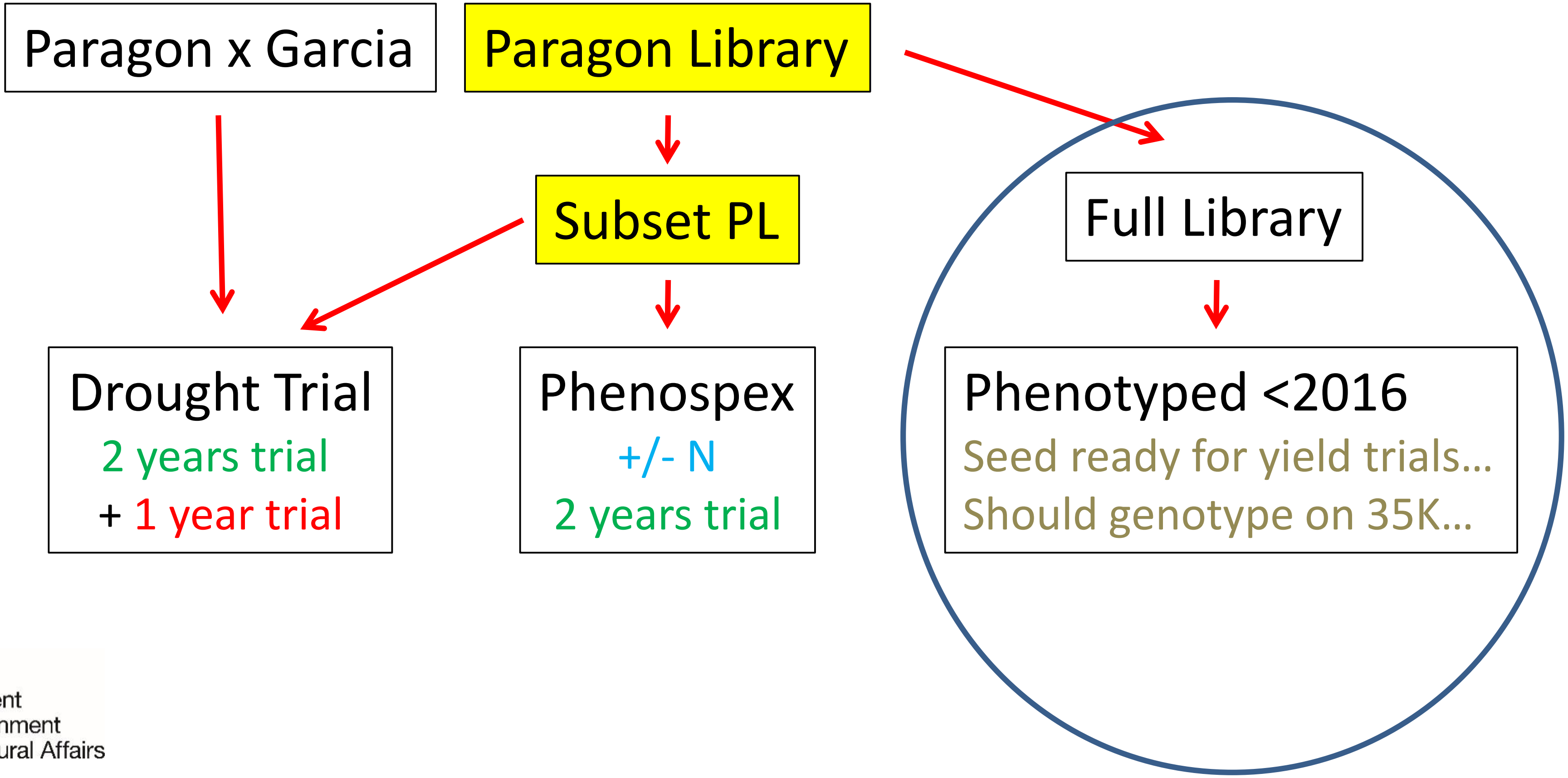
NILs in the genetic background of Paragon:
Rht-D1, Rht-B1, Rht8, Ppd-B1, Ppd-D1, Lr19, 1BL.1RS, 8 eps QTL, *Vrn1, Vrn3*, 3N, grain shape QTL, QTL from the Watkins collection (BBSRC WISP), selected WGIN mutants.



Paragon x Garcia

Paragon Library

Subset PL

Full Library

Drought Trial
2 years trial
+ 1 year trial

Phenospex
+/- N
2 years trial

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

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1. Dissecting UK drought tolerance in Paragon x Garcia

- Drought Trial repeated 2016-2017 (and 2017-2018!)
- 177 PxG RILs, Paragon and Garcia
- Includes subset of Paragon Library (Ppd, Rht, Staygreen and 7DL introgression)

- Riverside Field (2016-17) and Football Field (2017-2018) Church Farm: fields with light, sandy soil and suitable slope.
- Soil water content and aerial monitoring

- Traits measured: Stage 31, booting, DTEM, height, yield, specific weight, TGWT*
- Other traits investigated: waxiness, awns, ear compactness, tillering

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1. Dissecting UK drought tolerance in Paragon x Garcia

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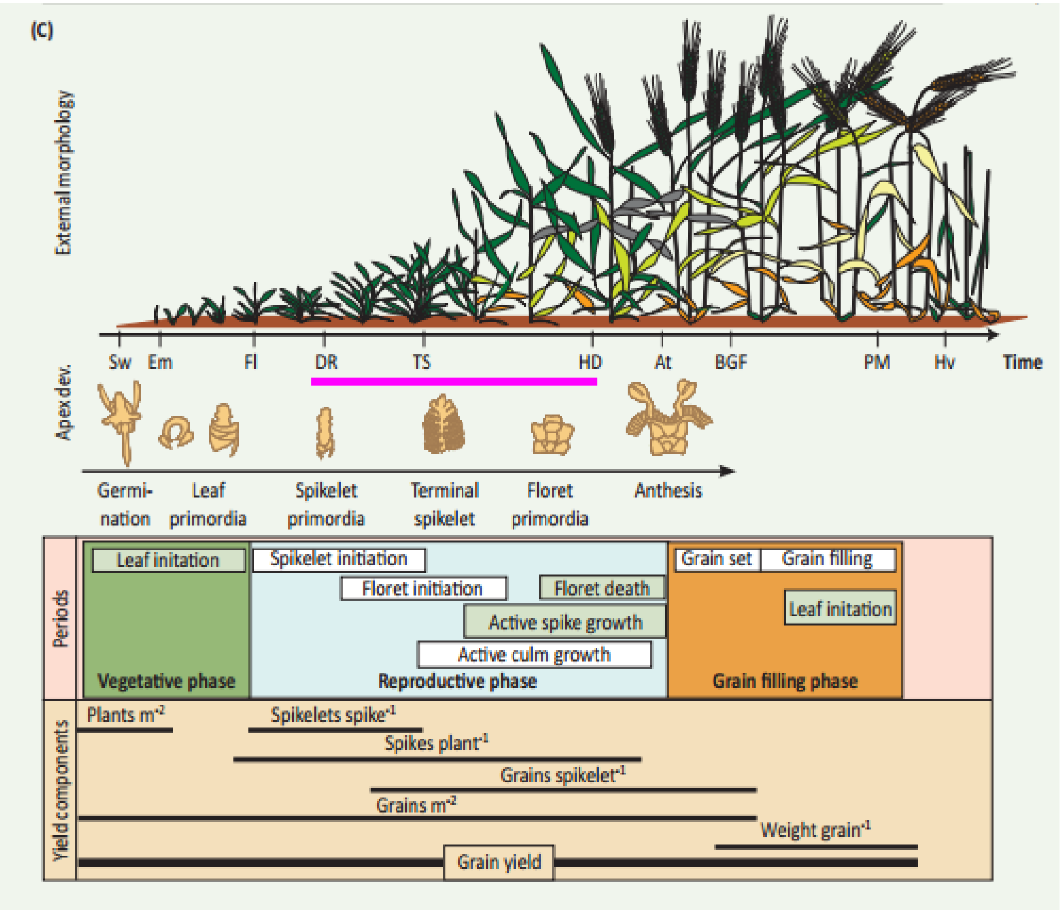
Stage 31



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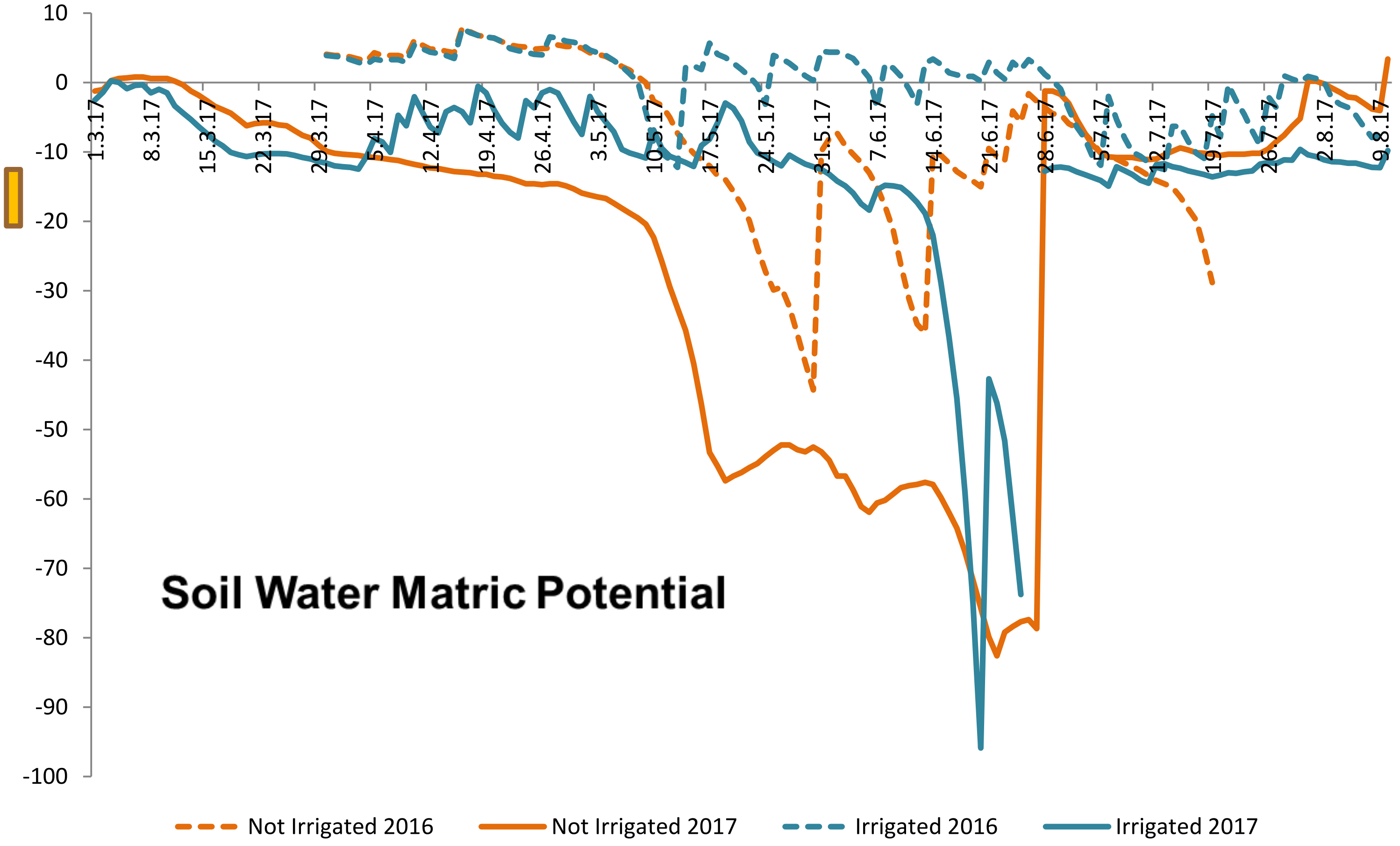
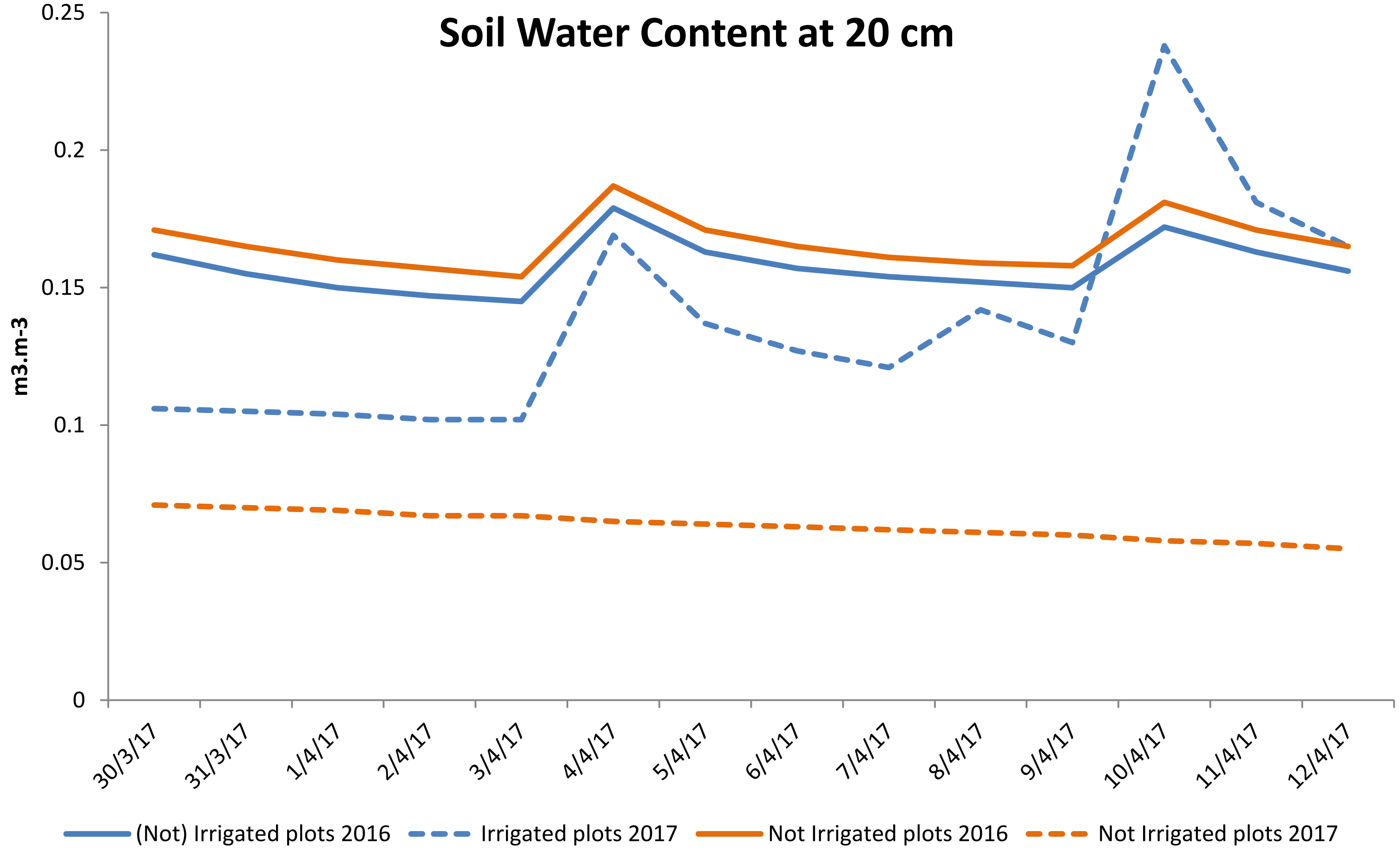
CIMMYT

1. Dissecting UK drought tolerance in Paragon x Garcia



- At potential of −33 kPa (−10 kPa for **sand**) soil is at **Field Capacity**
- Field Capacity is viewed as optimal condition for plant growth and microbial activity. ▮ - suggested Field Capacity for this trial

1. Dissecting UK drought tolerance in Paragon x Garcia



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1. Dissecting UK drought tolerance in Paragon x Garcia

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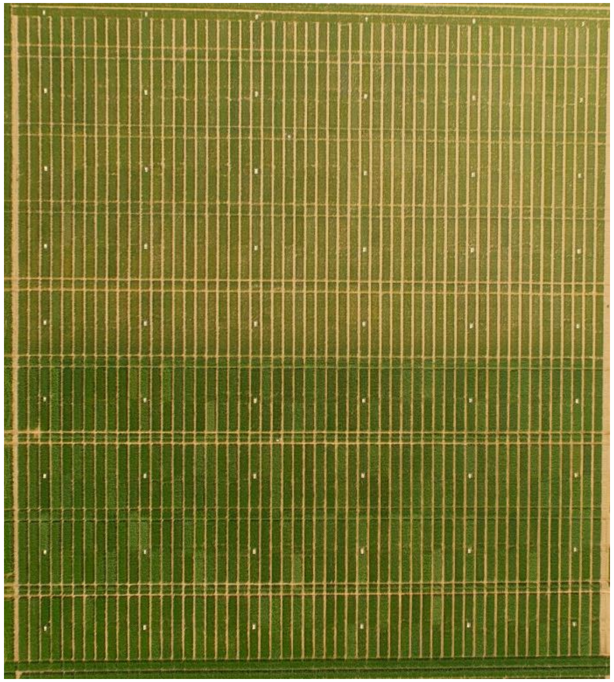
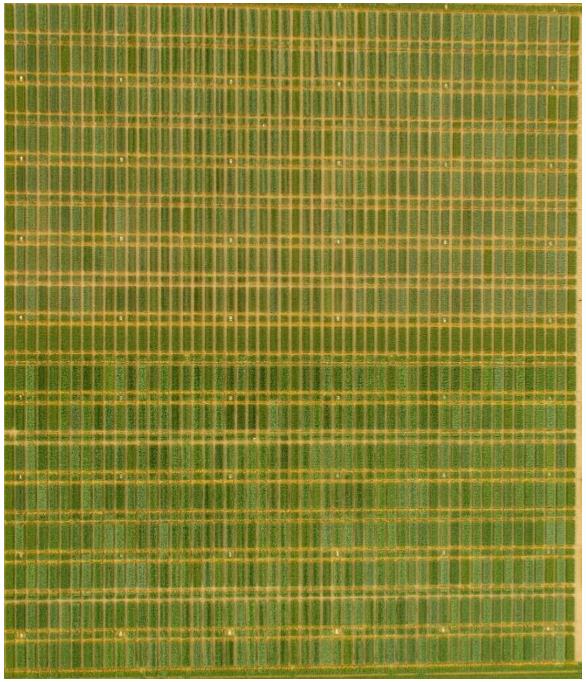
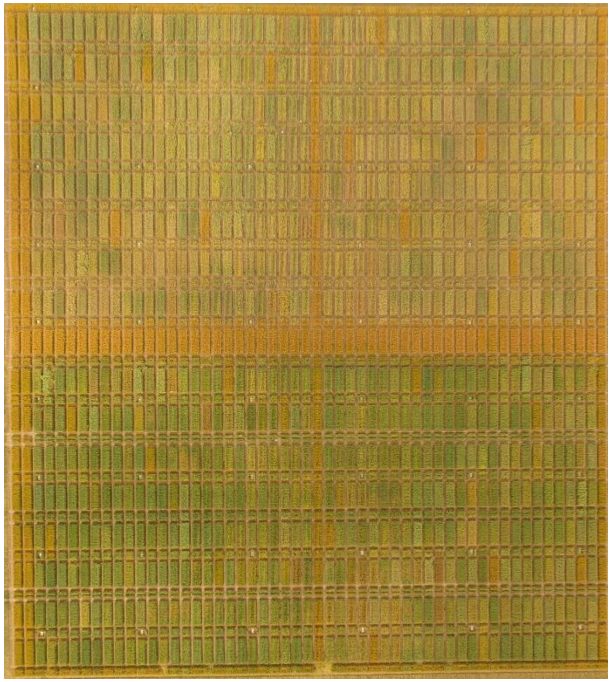
UAV imaging



11/05/2017



01/06/2017



30/06/2017

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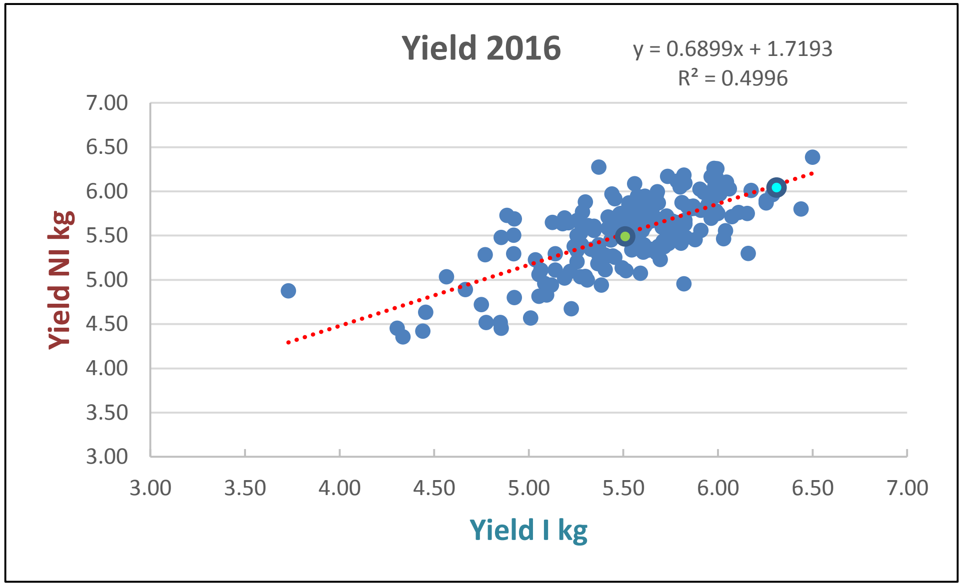
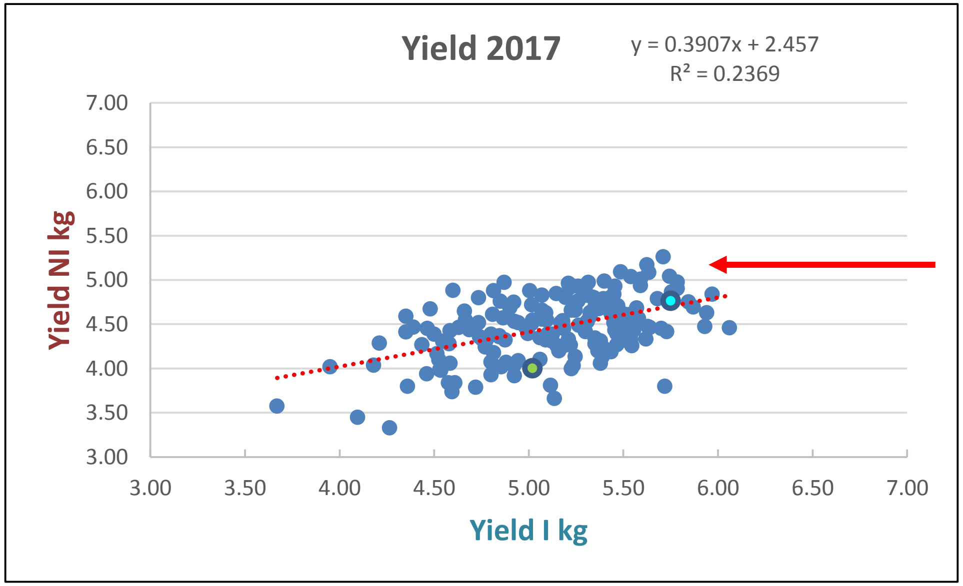
1. Dissecting UK drought tolerance in Paragon x Garcia





● Garcia
● Paragon

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1. Dissecting UK drought tolerance in Paragon x Garcia

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	2016				2017			
	Locus #	Linkage group	%Expl. Var	High allele	Locus #	Linkage group	%Expl. Var	High allele
Booting NI	178	2B	9.993	Garcia	178	2B	9.284	Garcia
	203	2D	21.515	Paragon	203	2D	21.223	Paragon
	402	4D	6.416	Garcia				
					494	5B	10.996	Paragon
	687	7A	17.044	Paragon	687	7A	10.762	Paragon
Booting I	178	2B	9.464	Garcia	193	2B	6.202	Garcia
	203	2D	14.91	Paragon	204	2D	53.926	Paragon
	688	7A	19.699	Paragon	687	7A	14.873	Paragon
DTEM NI	178	2B	10.253	Garcia	178	2B	6.55	Garcia
	204	2D	22.262	Paragon	203	2D	21.318	Paragon
					494	5B	11.974	Paragon
	687	7A	17.142	Paragon				
DTEM I	178	2B	9.849	Garcia	193	2B	4.727	Garcia
					204	2D	38.401	Paragon
	688	7A	15.3	Paragon	690	7A	21.061	Paragon
Height NI	16	1A	4.39	Garcia	16	1A	4.794	Garcia
	286	3B	3.652	Garcia				
	401	4D	62.258	Paragon	401	4D	62.299	Paragon
Height I	16	1A	6.365	Garcia	16	1A	5.068	Garcia
	292	3B	3.952	Garcia	292	3B	3.914	Garcia
	401	4D	59.611	Paragon	401	4D	59.277	Paragon
					700	7A	5.423	Paragon
Yield NI					173	2B	17.717	Garcia
	743	7B	16.81	Garcia				
Yield I	NO QTLs DETECTED				18	1A	11.358	Garcia
					173	2B	16.986	Garcia
Specific Weight NI	177	2B	8.997	Garcia	145	2B	11.341	Garcia
	232	3A	6.054	Paragon				
	403	4D	29.213	Paragon	402	4D	33.676	Paragon
	450	5A	2.47	Garcia				
					781	7D	5.234	Garcia
Specific Weight I					20	1A	5.003	Garcia
					173	2B	9.408	Garcia
	401	4D	38.147	Paragon	401	4D	33.018	Paragon
	441	5A	6.766	Paragon				
	455	5A	6.978	Garcia				

		Locus no.	Linkage group	%Expl. Var.	High value allele
Ear Compactness	NI	417	5A	16.776	Garcia
		480	5B	28.573	Paragon
	I	NO QTLs DETECTED			
Length awns	NI	585	6A	48.047	Garcia
	I	NO QTLs DETECTED			

Waxiness
Tillering

Stage 31 (1 rep only)

Locus name	%Expl. Var.	High value allele
BS00090234_2B	12.140	Garcia
BS00054733_5A	12.258	Paragon

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

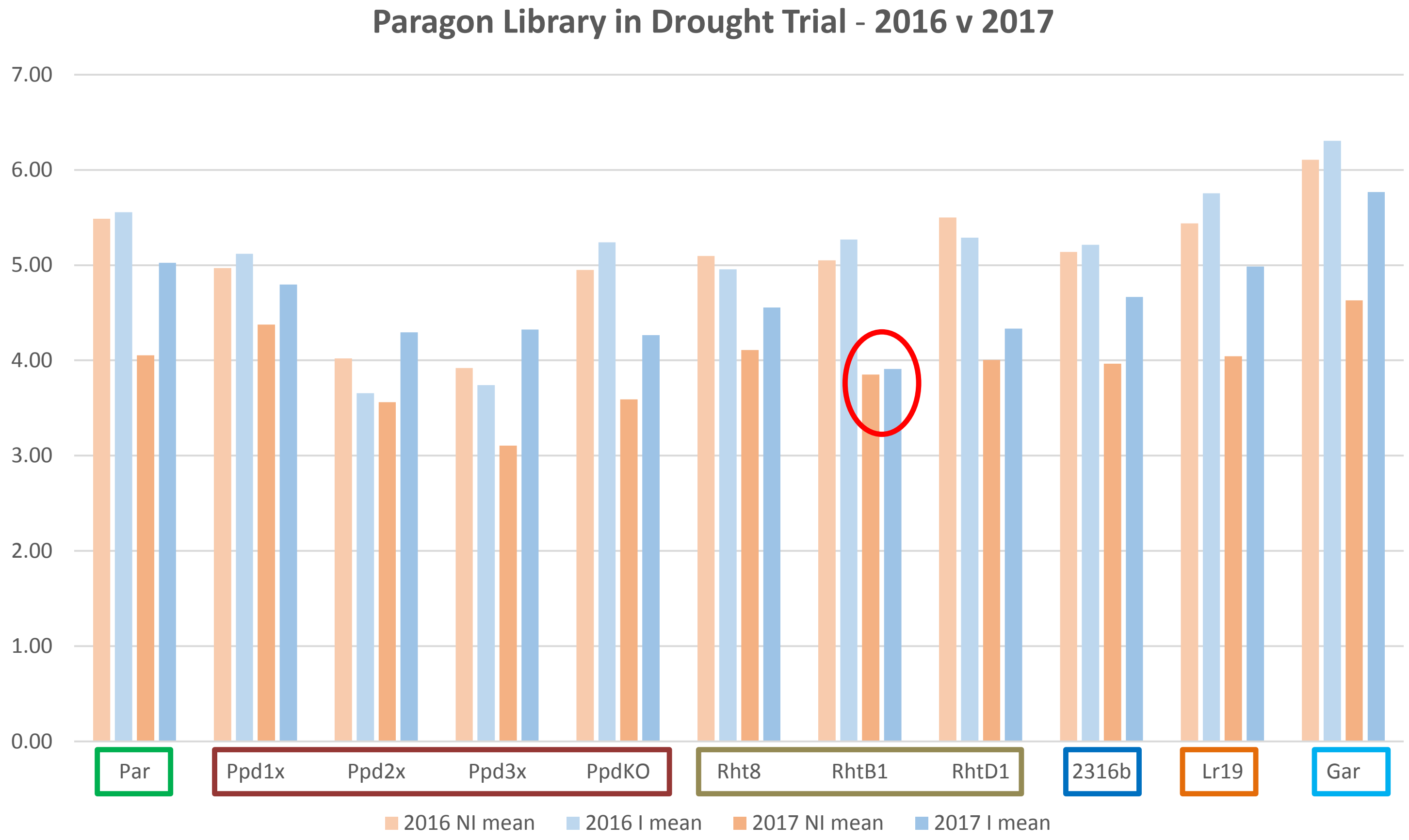
Subset of Paragon Library

Paragon	
Lr19 Kamb1	7DL 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

- Drought Trial
- Phenospex

*from *Agropyron elongatum*

2. Paragon NIL library



- Yields higher in 2016 than 2017
- Little difference in yields between NI and I in 2016
- Large effect on yield between NI and I in 2017 for most lines

2. Paragon NIL library

- Subset PL lines drilled under Phenospex (2015-2016/2016-2017)
  - Two randomised blocks of 16
  - One treatment – all plots + nitrogen (40 kg/h)
  - Two subsequent treatments +/- additional nitrogen (250 kg/h)

Had reasonable results last year but this years results difficult to interpret due to heavy sampling and drought conditions.

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2. Paragon NIL Library

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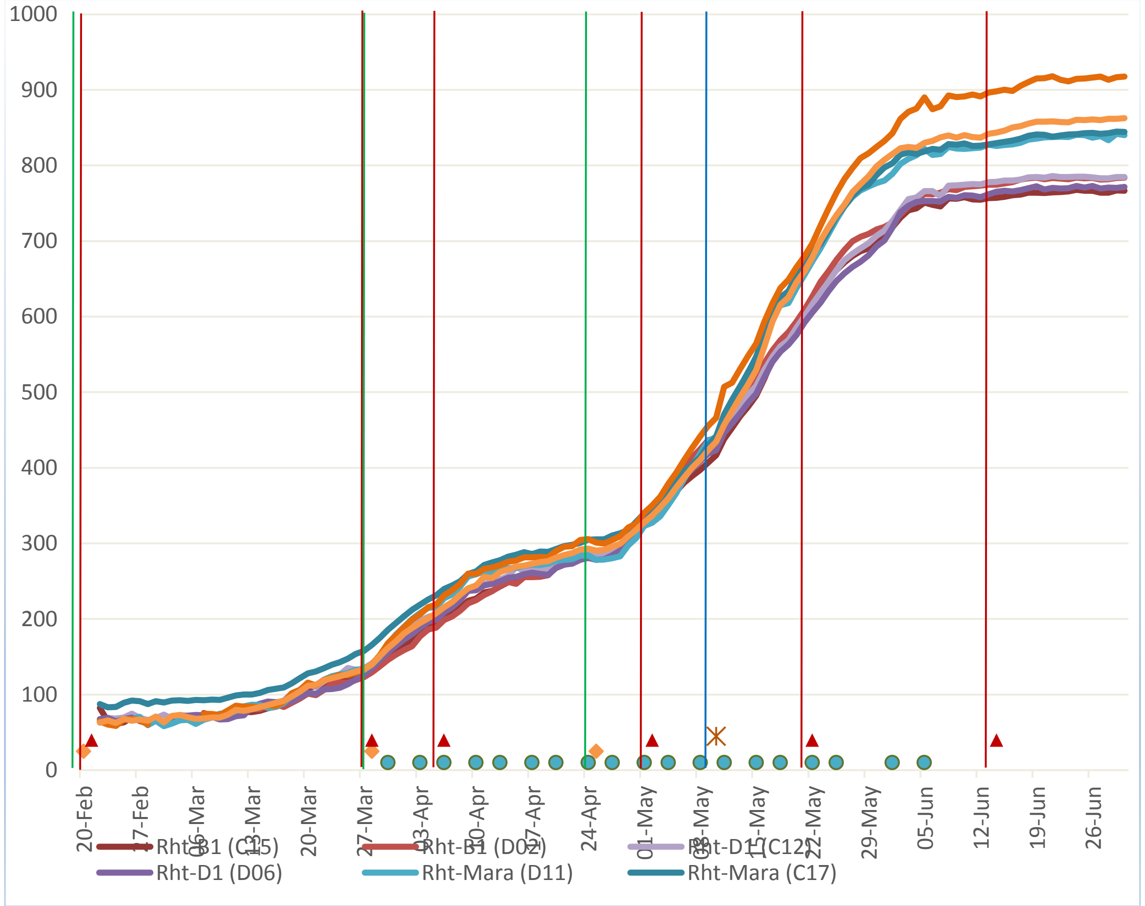

- Collaboration with Ji Zhou's group (EI)
- Phenospex measurements show time when growth rates of Paragon/Rht8 diverge from RhtB1/RhtD1
- Sample collection and RNA analysis

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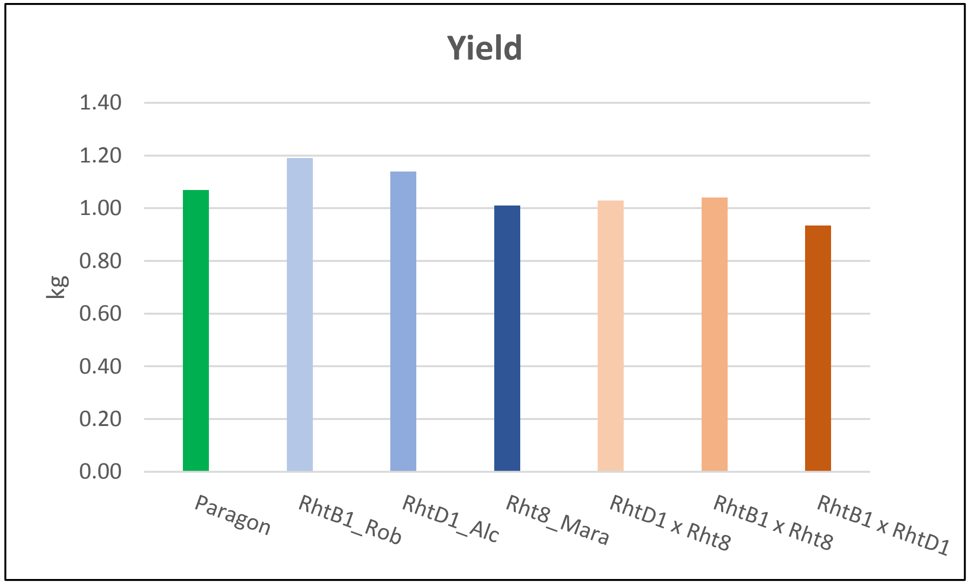
3. Informing multiple marker assisted selection for yield stability using the Paragon library

- Lines generated to test this:
- *RhtB1* x *Rht8*
- *RhtD1* x *Rht8*
- *RhtB1* x *RhtD1*
- 1 m plots 2016-17



RhtB1+RhtD1 *Rht8+RhtD1*

Rht8+RhtB1 Paragon

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

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

Autumn drilling of yield trial

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

- In addition a 'winter Paragon' was produced by crossing in *VrnA1* + *VrnB1* from Malacca – *see increased yield with winter Paragon* (Simon Orford)
- Subsequently crossed into *Rht8* / *RhtB1* / *RhtD1* to produce winter, semi-dwarf Paragon
- Three genes segregating… need to screen in glasshouse

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WGIN3 Projects: Avalon and Cadenza

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

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- 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

- Produced ‘Graphical Genotypes’ for all 21 chromosomes
- Selected lines to give the optimal tiling path for each chromosome
- Do not have complete coverage of genome

- F2 progeny of crosses to recurrent parent of selected lines to be genotyped with BS markers to determine the presence of required segment(s) [2018]
- 94 individuals, from 60 selected NILs =~6000 DNA extractions
- Individually chosen sets of ~ 25 KASP markers for each NIL

- Seed of these lines will be bulked and made available to the community

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

Simon Orford

- All crosses at F2
- Populations in pink at F4 (-> F5/F6) aiming for ~400 lines each
- Populations in yellow at F3 (-> F5/F6) aiming for ~400 lines each

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.

Cross	**Parent 1**	**Parent 2**	
Cross 1	DH109	DH160	**F2 progeny only from 1 plant**
Cross 2	DH61	DH182	**F2 progeny from many plants**
Cross 3	DH27	DH61	**F2 progeny from many plants**

- F2 lines need to be genotyped but lack resources to do this…

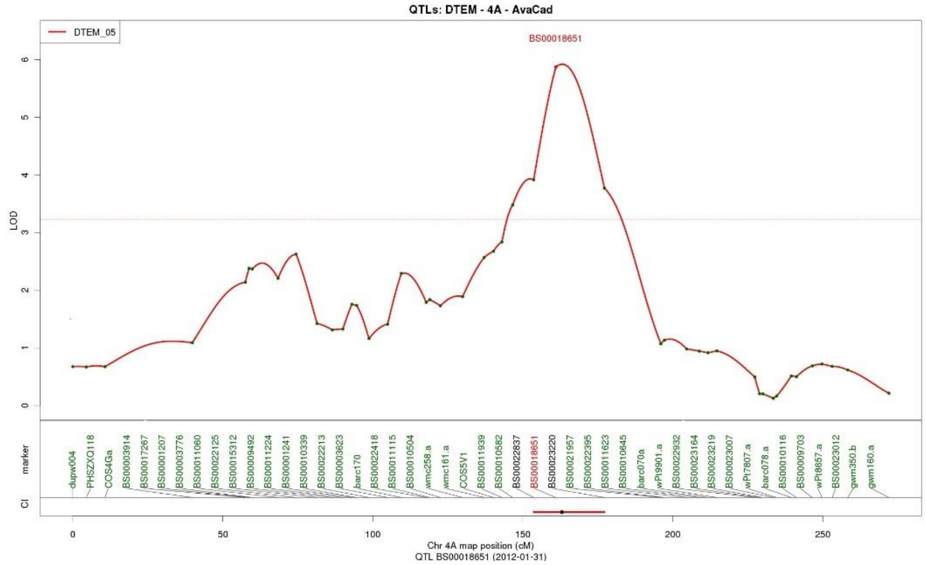
WGIN3 Projects: Additional Populations

- 3N alien introgression (from *Aegilops uniaristata*) into CS
- Shows aluminium tolerance: Al toxicity primarily affects the division and elongation of the root apex
- 3N line shows prolific root phenotype

- Three different recombinant lines (Rec4-1, Rec 5-1 and Rec 6-3)
- Crossed to winter elite lines – Cordiale, Napier and Robigus
- Difficult to get markers to follow introgressed region…
- … few homozygous lines identified
- Autumn drilling of multiplication plots from Rec5-1 x Cordiale

WGIN3 Projects: Additional Populations

- QTL on 4A for DTEM in AxC NILs
- Only identified when Axiom mapping data gave better coverage of chromosome 4A
- Possibly PhyB



- Lines homozygous for the QTL region in an Avalon or Cadenza background have been identified
- Autumn drilling of multiplication plots

Update on Diversity trial 2017

Andrew Riche

WGIN management meeting Oct 2017

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BBSRC
bioscience for the future

Lawes
Agricultural
Trust

Activities

- Continued regular measurements – anthesis, senescence, grain and straw yield
- Extra Wgin 3 work – sampling at anthesis, measurement of mineral uptake during grainfill (8+ elements)
- Continued regular monitoring by UAV (21 occasions, Nov – July)

Used by others as a resource:

- George Savill, PhD student, assessing grain protein distribution
- Dr Y Wan with Zhiqiang Shi (PhD student) low protein wheat quality (5 lines)
- Dr P Buchner with Anne Rossman (PhD student) investigating effect of applying late foliar N on 4 varieties

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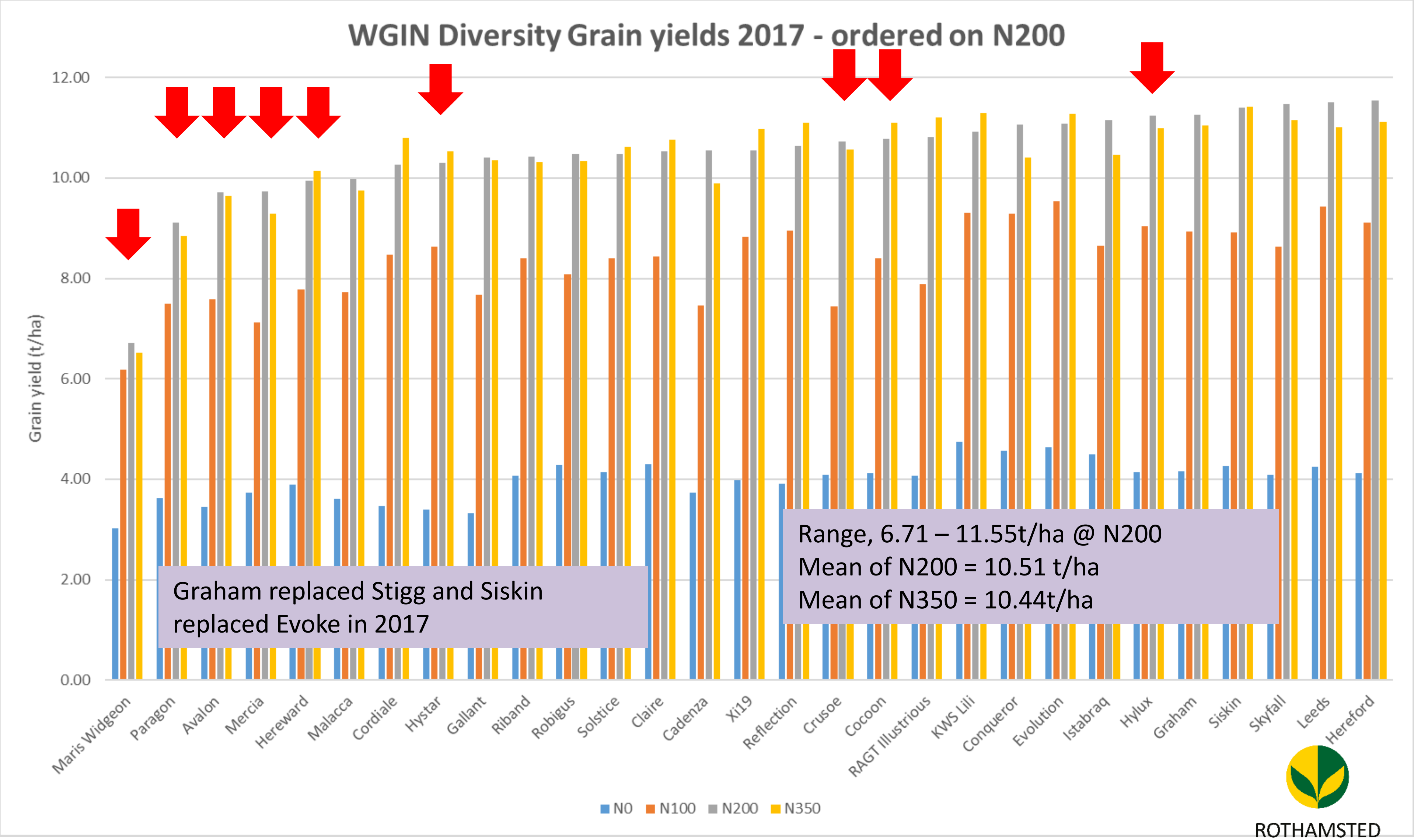
Growth and yield, 2017



Graham replaced Stigg and Siskin replaced Evoke in 2017

Range, 6.71 – 11.55t/ha @ N200
Mean of N200 = 10.51 t/ha
Mean of N350 = 10.44t/ha

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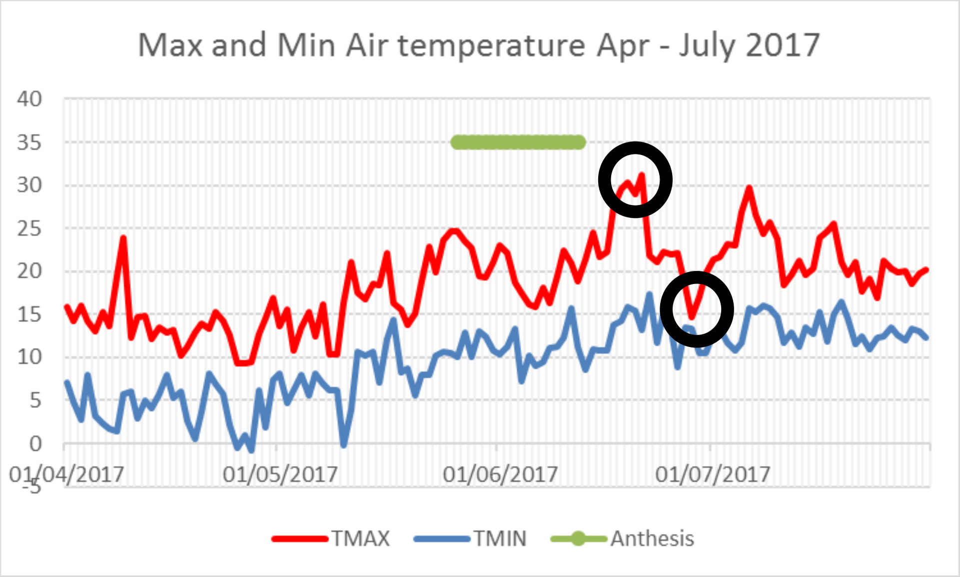
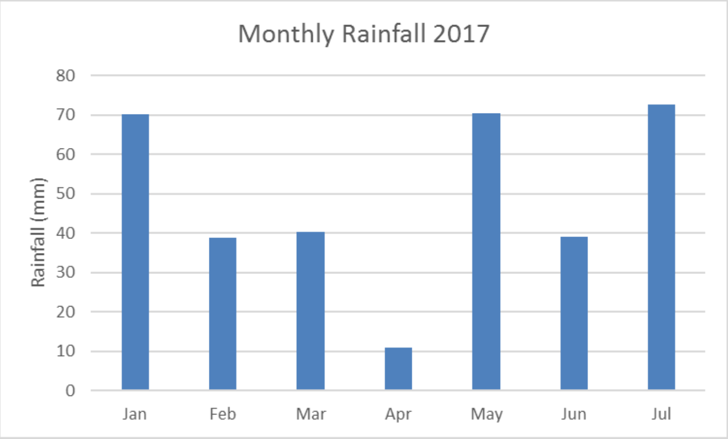
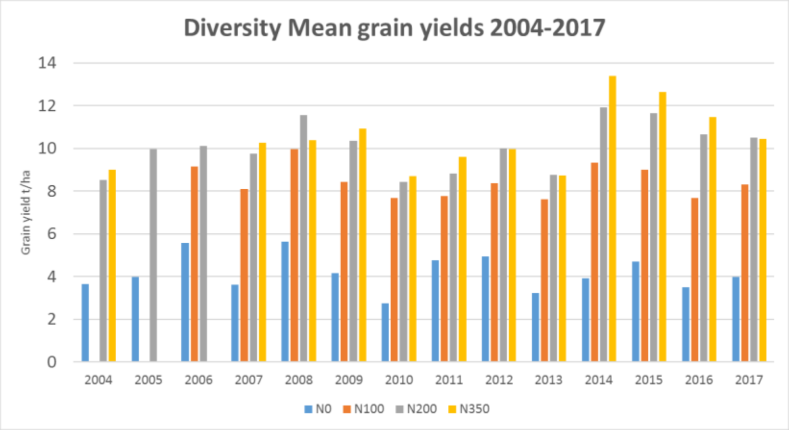
Growth and yield, 2004-2017



Anthesis occurred between 26th May and 12th June

21/6/17 31.3^{0}C
28/6/17 14.7^{0}C





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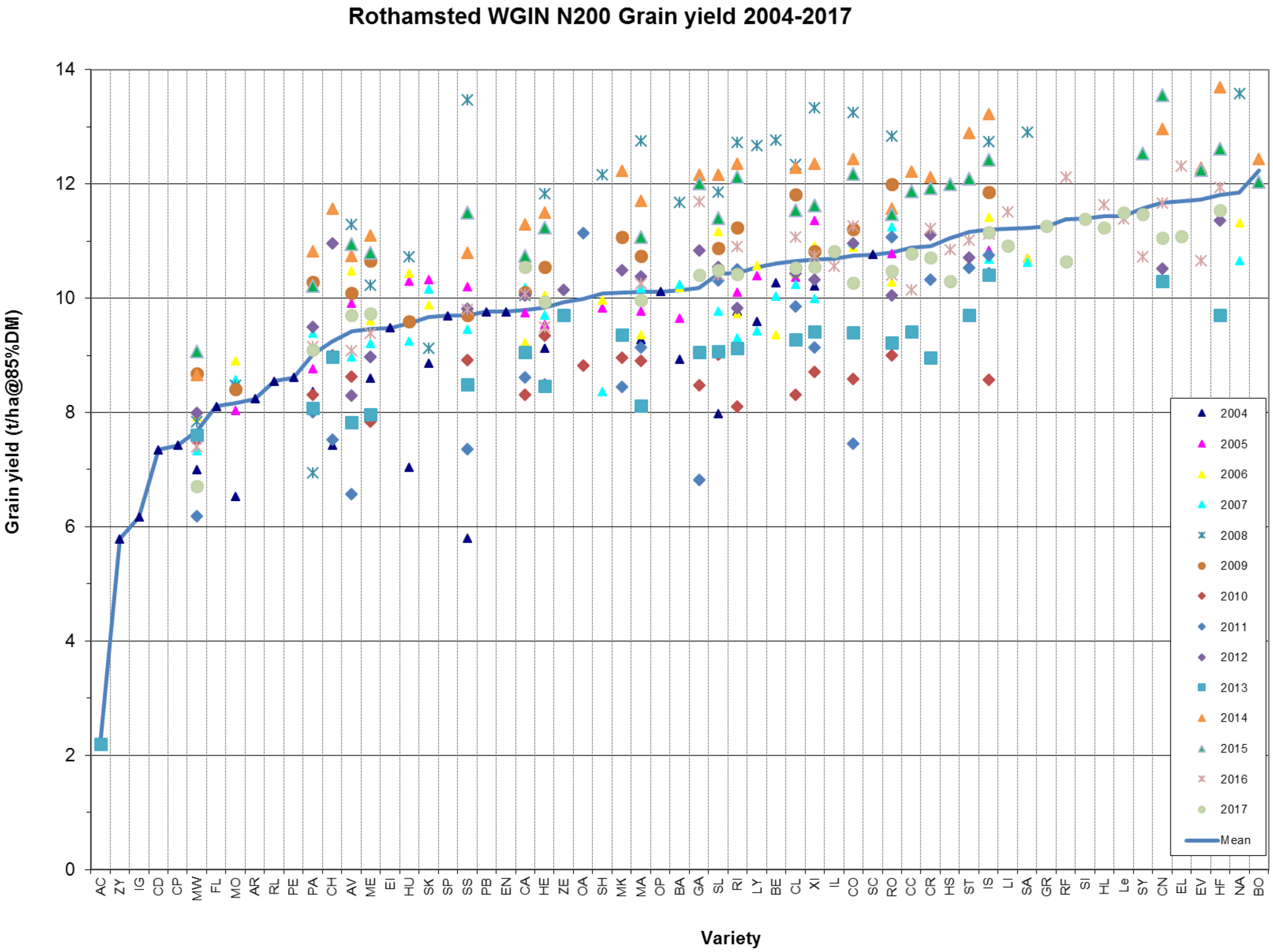
Growth and yield, 2004 - 2017



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2018 Diversity trial

- Barrel (highest yielding Gp3) replaces Cocoon
- Zyatt (highest yielding Gp 1) replaces Gallant

- Seed ready, should be drilled next week

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UAV activities

- 2017 images not yet processed – priority for the autumn
- Some preliminary trials of disease ID from UAV images

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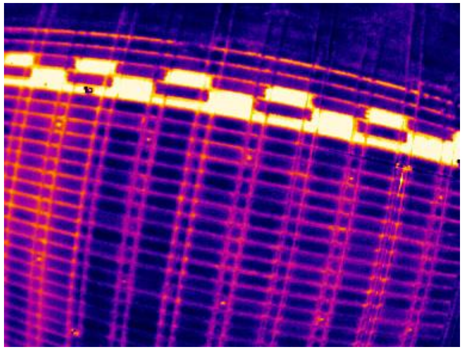
UAV Update

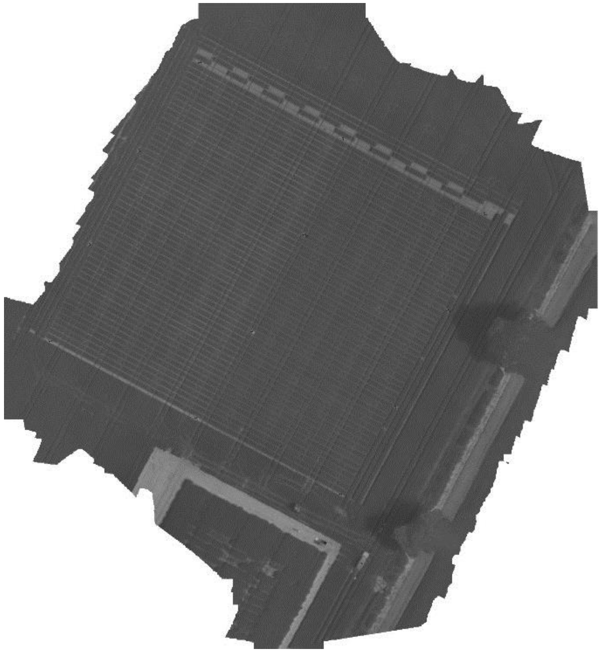
Ambient light sensor

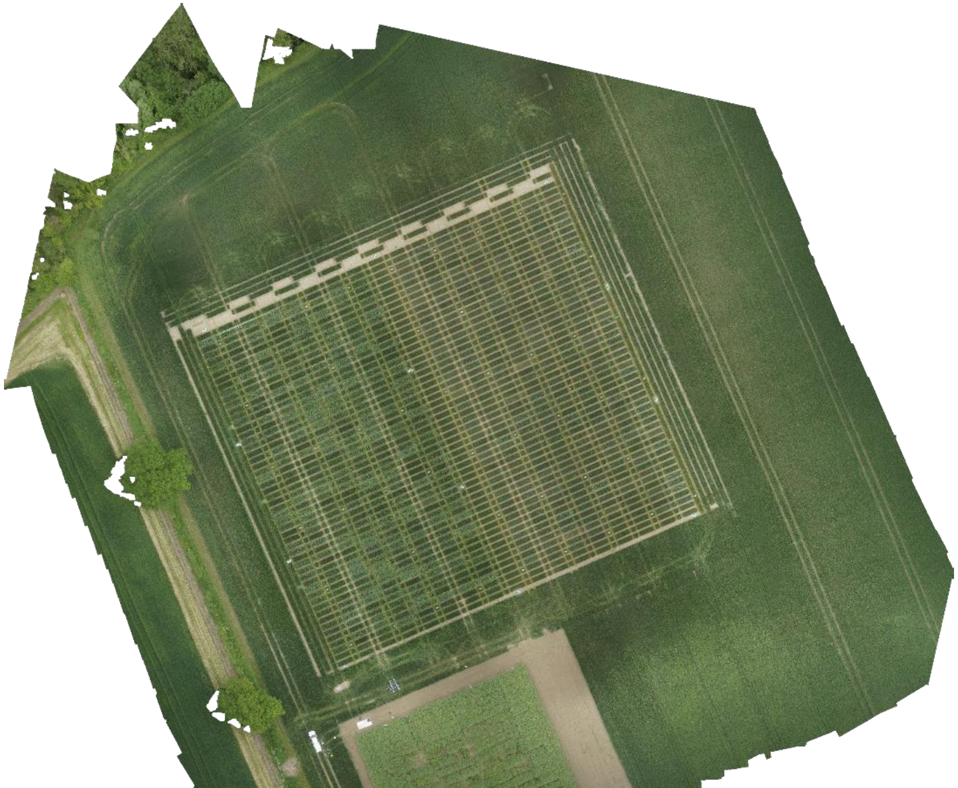
5m altitude with 25mm lens, 0.65mm/pixel
With 45mm lens, 0.37mm/pixel

RTK GPS Base station

Thermal imagery – JIC drought trial







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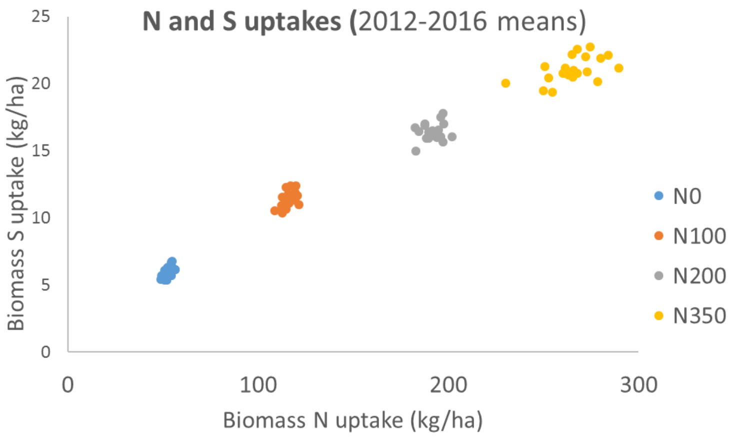
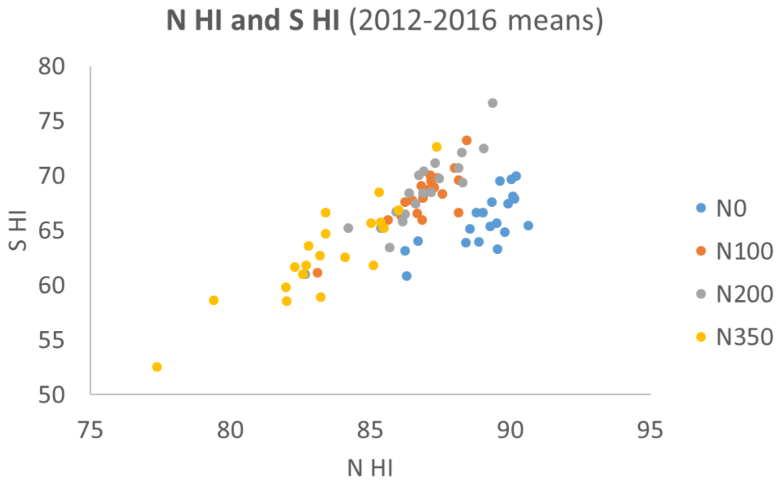
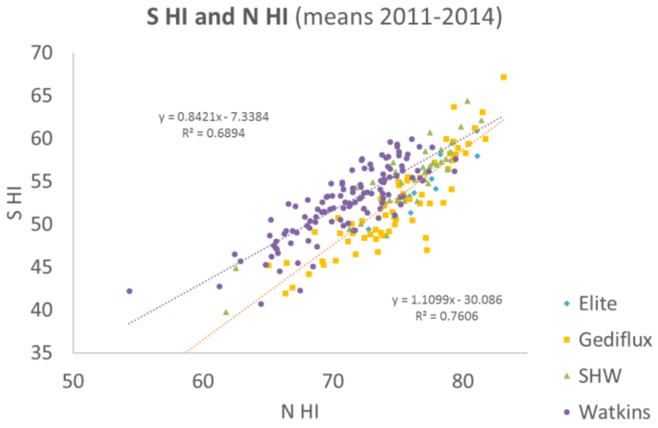
Minerals - S



Influence of N nutrition on total N and S uptake and partitioning in 20 modern wheats



Modern material lower SHI



Modern material better NHI

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Acknowledgments

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WGIN3 Management Meeting

6th October 2017

Screening germplasm for resilience to aphids (WP2.3)

Gia Aradottir

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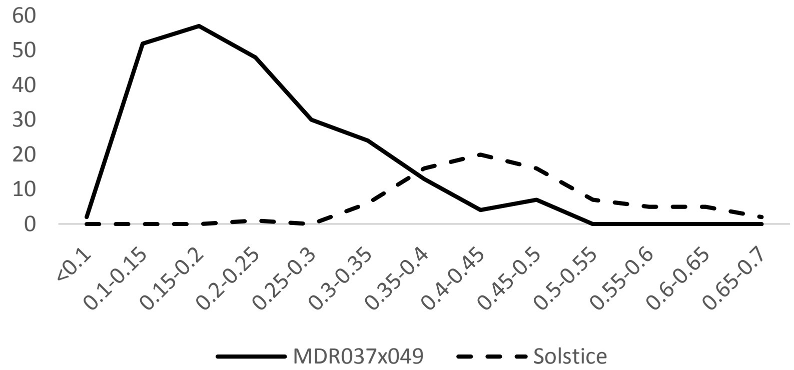
Screening germplasm for resilience to aphids (WP2.3)

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

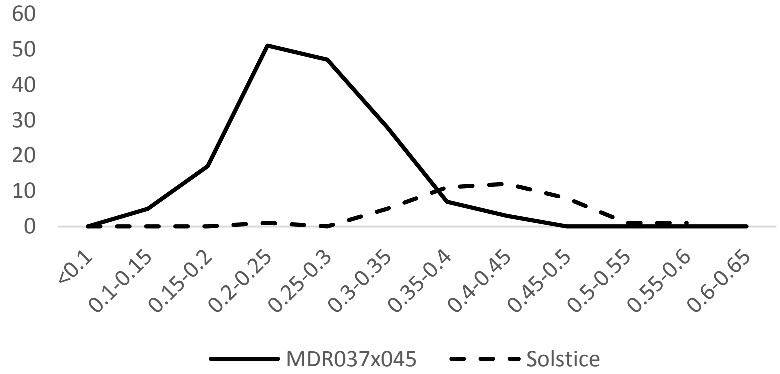
Crosses of *T. monococcum* lines made by Mike Hammond-Kosack: MDR037 (susceptible) x MDR045, MDR049 and MDR657 (all showing evidence of partial resistance)

F1, F2 and F3 generations of these crosses have now been tested in the phenotyping screen against both aphid species. F3s of each cross tested included plants with the best and the worst effective partial resistance to the aphid species.

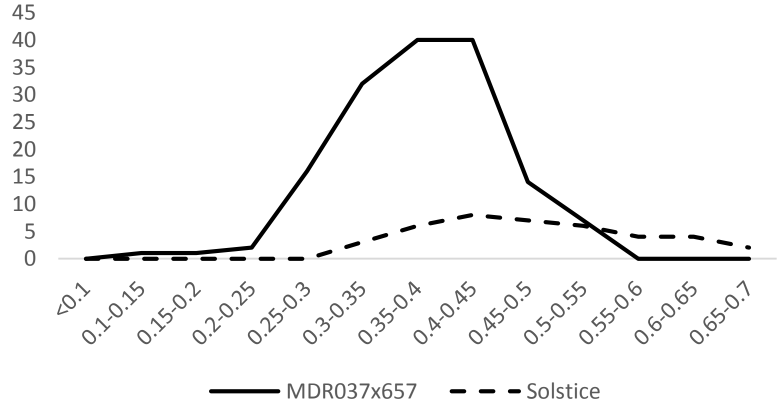
S. avenae on *T. monococcum F3* crosses of MDR37 x 49

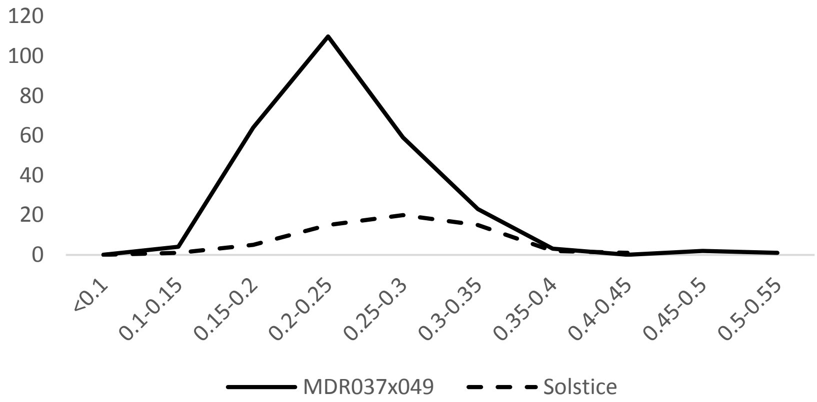


S. avenae on *T. monococcum* F3 crosses of MDR37 x 45

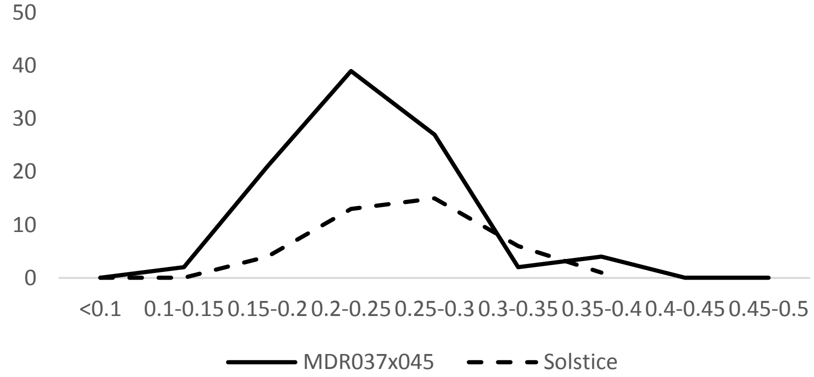


S. avenae on *T. monococcum* F3 crosses of MDR37 x 657

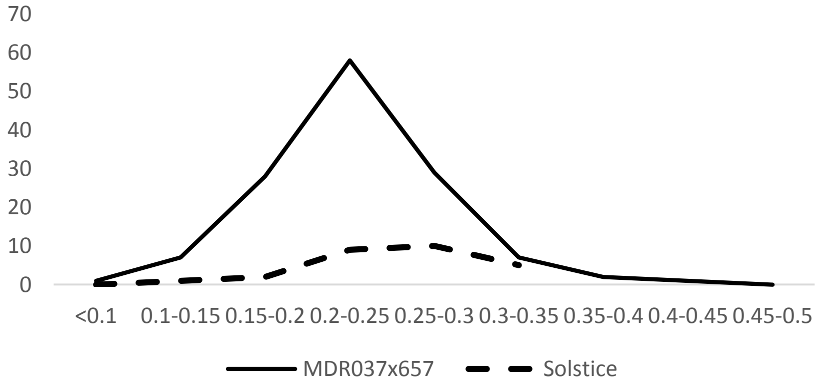




R. padi on *T. monococcum* F3 crosses of MDR37 x 49



R. padi on *T. monococcum* F3 crosses of MDR37 x 45



R. padi on *T. monococcum* F3 crosses of MDR37 x 657

Screening germplasm for resilience to aphids (WP2.3)

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

ROTHAMSTED RESEARCH

- F3 generation has been harvested
  - Need threshing and decisions made on further phenotyping

- Tissue samples collected and waiting to be tested

Department for Environment Food & Rural Affairs

Wheat Genetic Improvement Network

WGIN 3
Resistance to foliar diseases

Vanessa McMillan
Kim Hammond-Kosack

WGIN MM 6th October 2017

Resistance to multiple foliar diseases

ROTHAMSTED
RESEARCH

Watkins 2008 Field Trial

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

			2008 Disease assessments			
Accesssion	Growth habit	Country of Origin	Yellow rust	Brown rust	Septoria	Mildew
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
262	Spring	Canary Islands	0	0	0	0
399	Spring	China	T	0	T	0
495	Spring	Morocco	0	0	T	0
610	Spring	Yugoslavia	0	0	T	T
733	Spring	Iran	T	T	T	T
786	Spring	USSR	0	T	T	0

0 – no disease , T = trace

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BBSRC
bioscience for the future

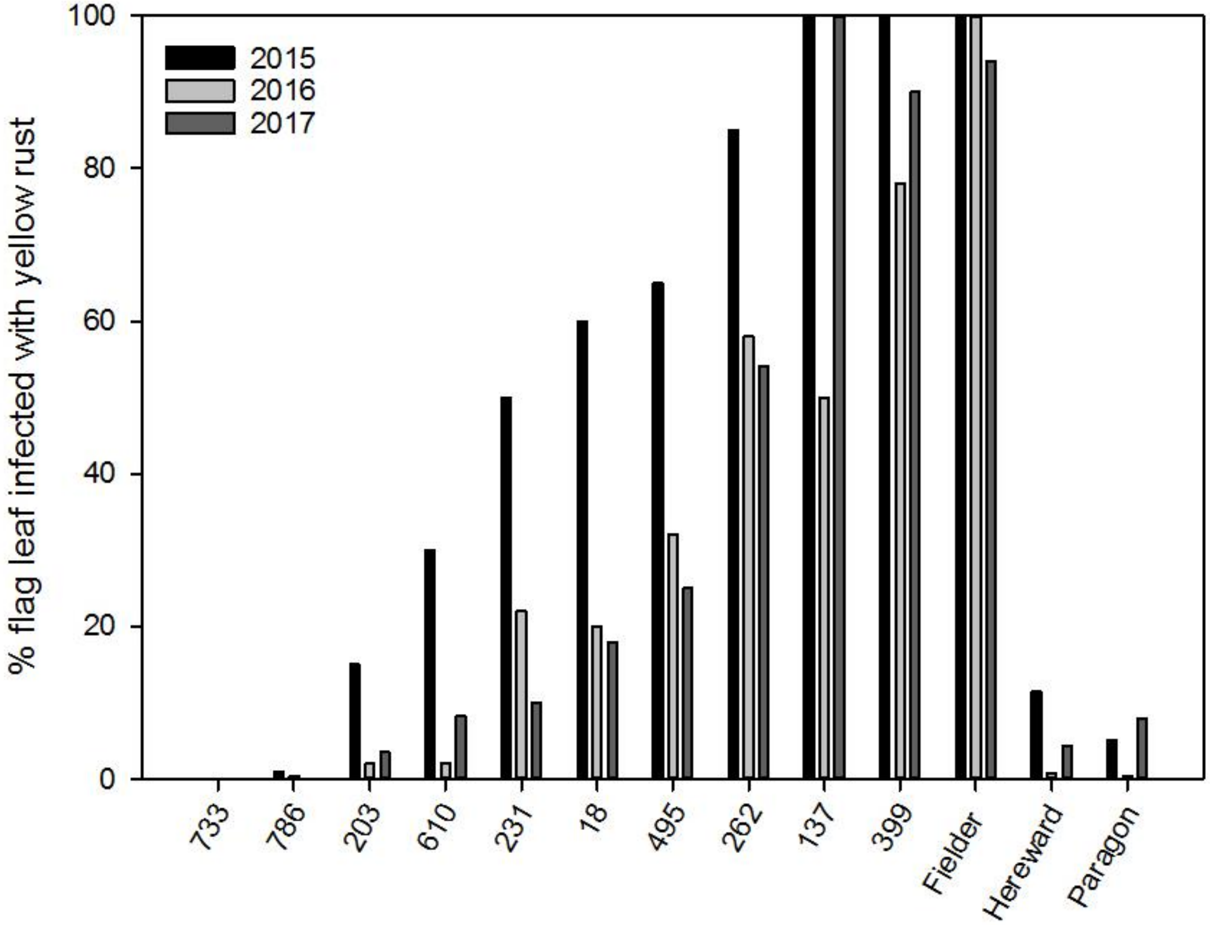
Watkins foliar disease field trials

1st and 3rd wheat replicated field trials

Three field seasons
2014-2015
2015-2016
2016-2017

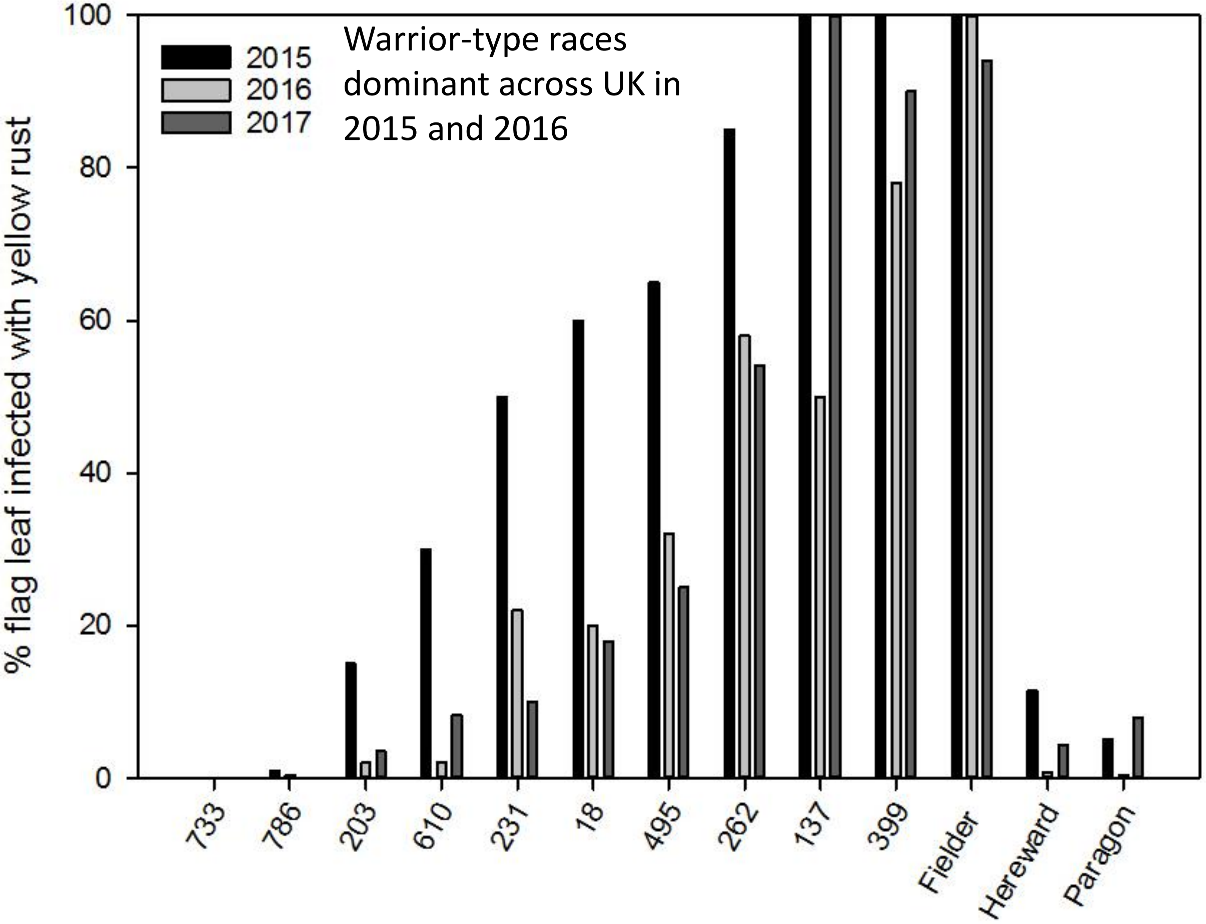
26th June 2015 1st wheat Long Hoos 4

Evidence of resistance to yellow rust



- Did susceptible Watkins lines escape disease in 2008 or change in YR races?
- Hereward and Paragon good source of resistance to current YR races

Evidence of resistance to yellow rust



Warrior-type races dominant across UK in 2015 and 2016

YR races

Year	Variety
2000	Robigus
2008	Solstice
2011	Sterling
2011	Warrior 1
2012	Warrior 4
2014	Kranich
2015	Invicta

2017 field trial – samples submitted to "Field Pathogenomics" pathogen surveillance programme

- Did susceptible Watkins lines escape disease in 2008 or change in YR races?

Watkins 137

Highly susceptible

100 % flag leaf disease severity on 24th June 2015

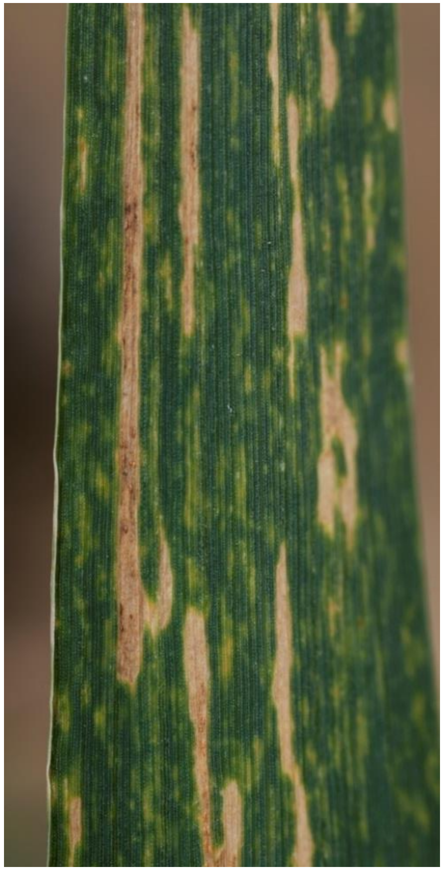
Watkins 733

Highly resistant
No yellow rust sporulation visible throughout whole field season

Watkins 203

Low levels of yellow rust sporulation

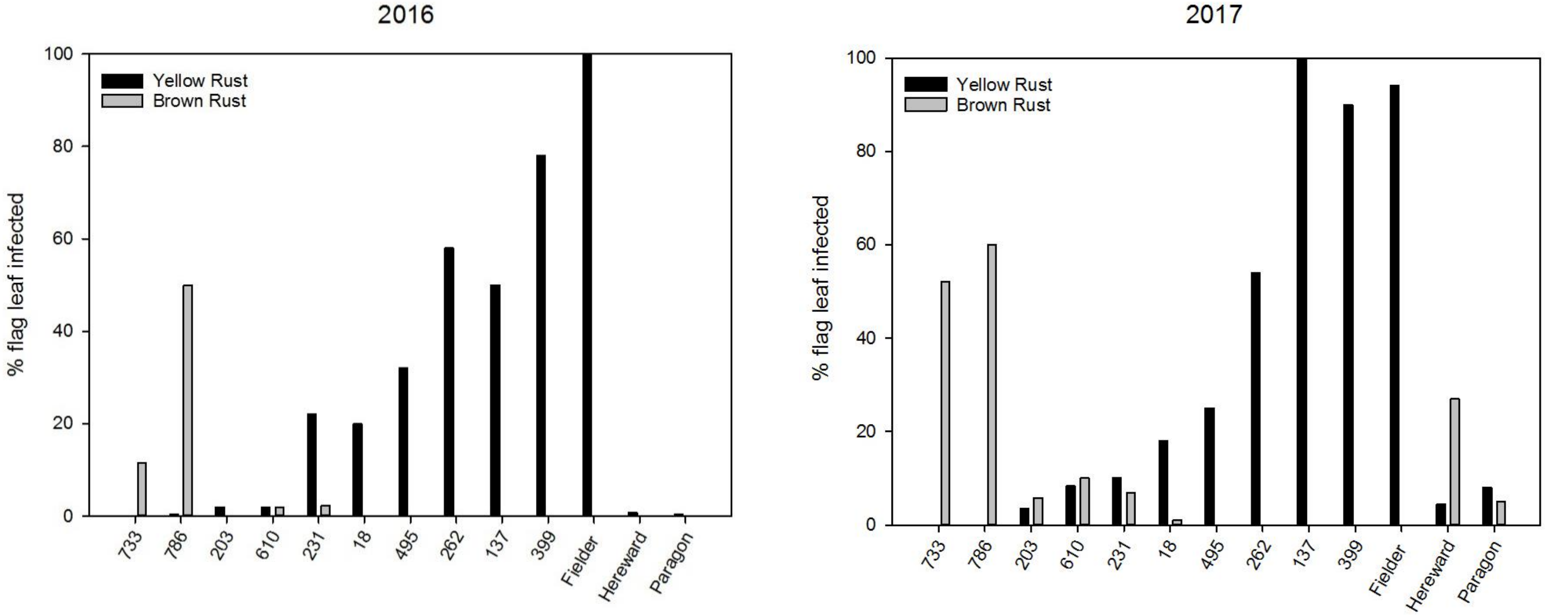
15 % flag leaf area infected with yellow rust on 24th June 2015



Paragon

Low levels of yellow rust sporulation
5 % flag leaf area infected with yellow rust on 24th June 2015
Large necrotic stripes

Brown rust on flag leaves

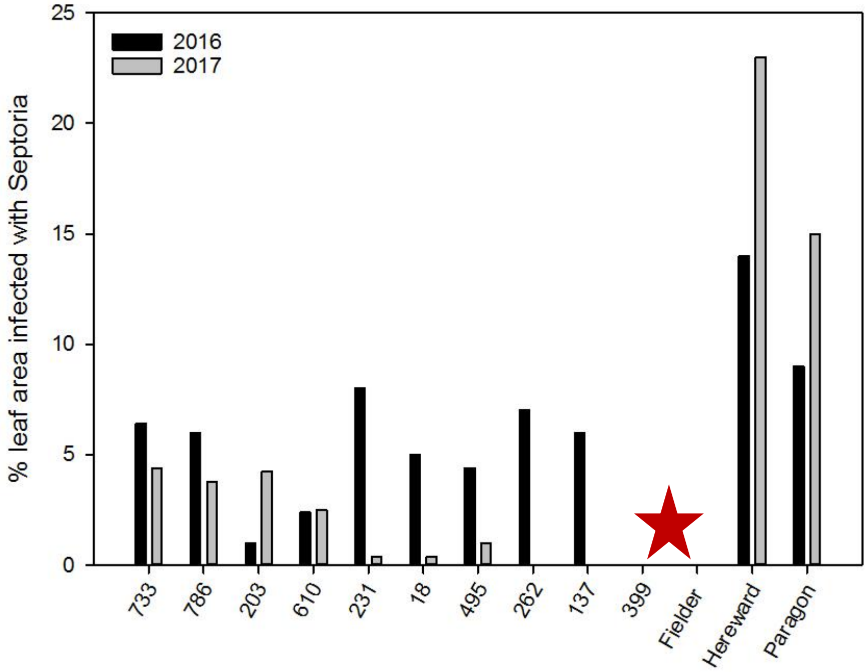


Watkins 786

- Watkins 733 and 786 were most resistant to yellow rust but are very susceptible to brown rust – do not possess multi disease resistance

- Difference in brown rust races between 2016 and 2017?

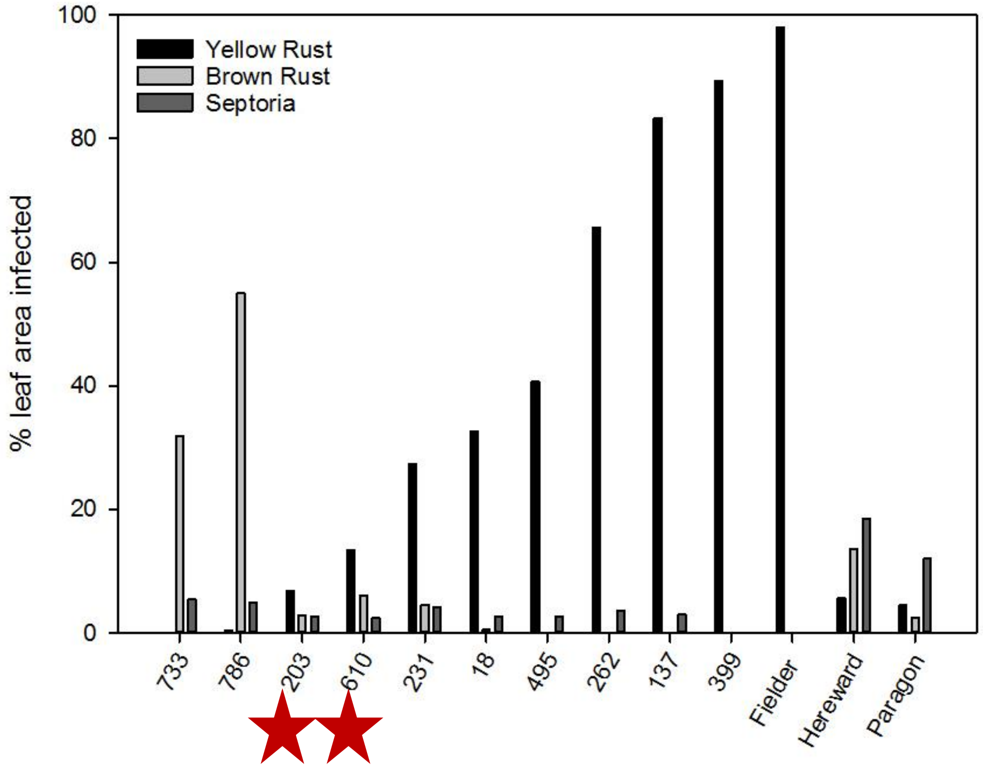
Septoria on lower leaves



★ Low levels of Septoria on all genotypes except 399 and Fielder where high levels of yellow rust are found, therefore impossible to assess Septoria infections

- All Watkins genotypes less susceptible to Septoria than Hereward and Paragon

Average disease scores across all field seasons



2014-2015 only	YR
2015-2016	YR, BR and S
2016-2017	YR, BR and S

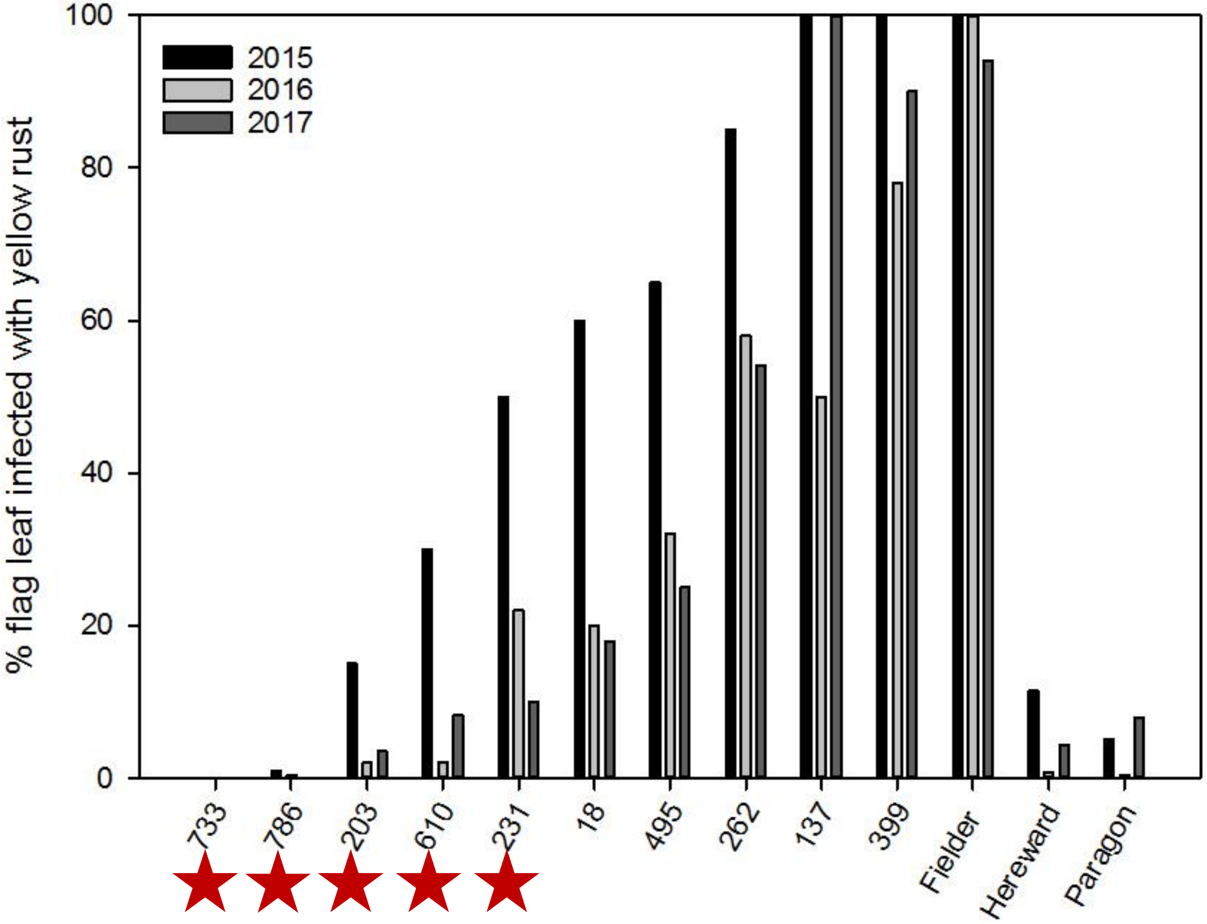
Septoria levels low across both field seasons

Very little powdery mildew across all three field seasons on wheats – not scored

★ Watkins 203 and 610 most promising for showing high levels of resistance against multiple foliar diseases

- Watkins 610 may be escaping disease through later leaf emergence

Evidence of resistance to yellow rust



★ Chosen for mapping population development

- Did susceptible Watkins lines escape disease in 2008 or change in YR races?

Watkins mapping population development

Summer 2015 Field crossing with cv. Fielder

Accession Number	Growth habit	Country of Origin	Ears crossed	F_1 grains
203	Winter	India	8	31
231	Spring	Hungary	8	54
610	Spring	Yugoslavia	6	33
733	Spring	Iran	6	49
★ 786	Spring	USSR	N/A	N/A

Summer 2016

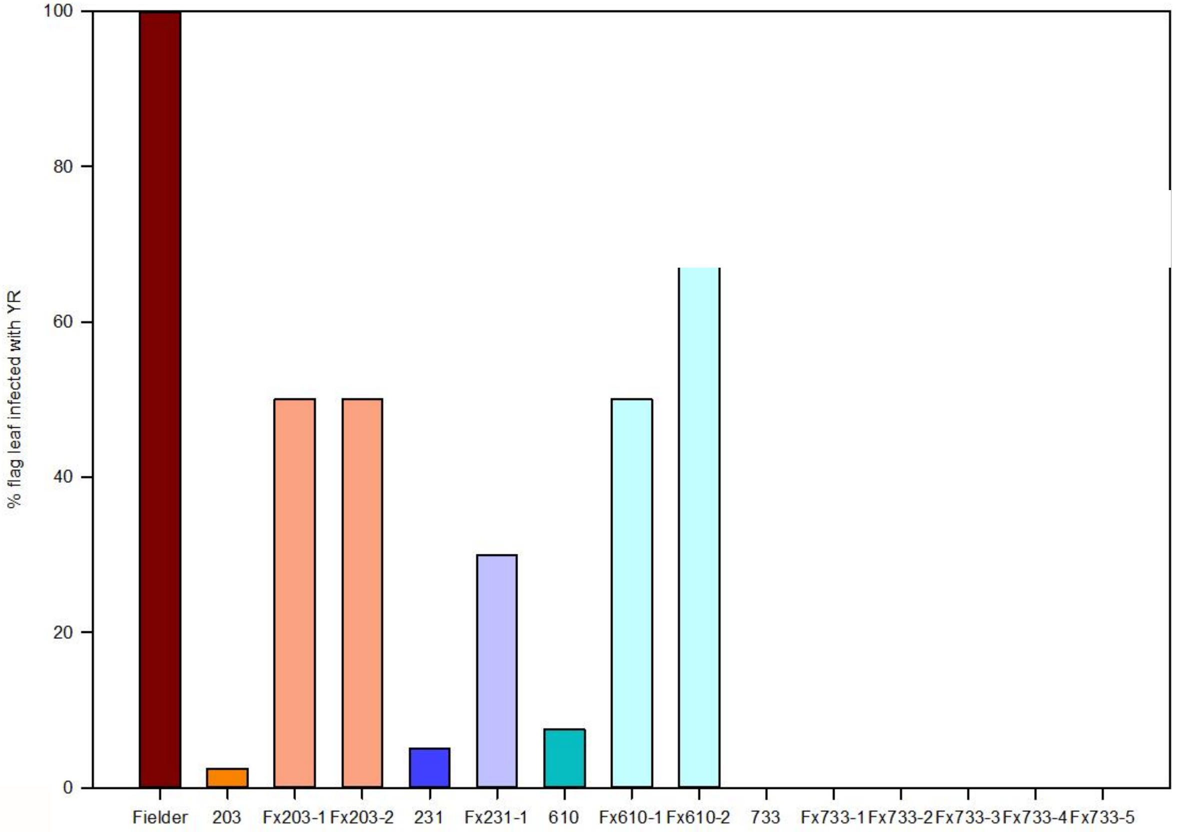
- F_1 grain sown in glasshouse to generate F_2 and for backcrossing to cv. Fielder
- Watkins 786 x Fielder crossing carried out in glasshouse at later date
- F_1 grain included in a spring field trial 2016 to study inheritance

★ Watkins 786 originally excluded from crossing because had high levels of yellow rust during tillering – possibly APR?

F_1 plants – spring field trial

- Six F_1 grain from each of the 4 crosses sown
- Two replicate plots of parent genotypes (40 seeds per plot)

- Yellow Rust assessments on 30th June 2016 – flag leaf assessments



F_1 plants – spring field trial

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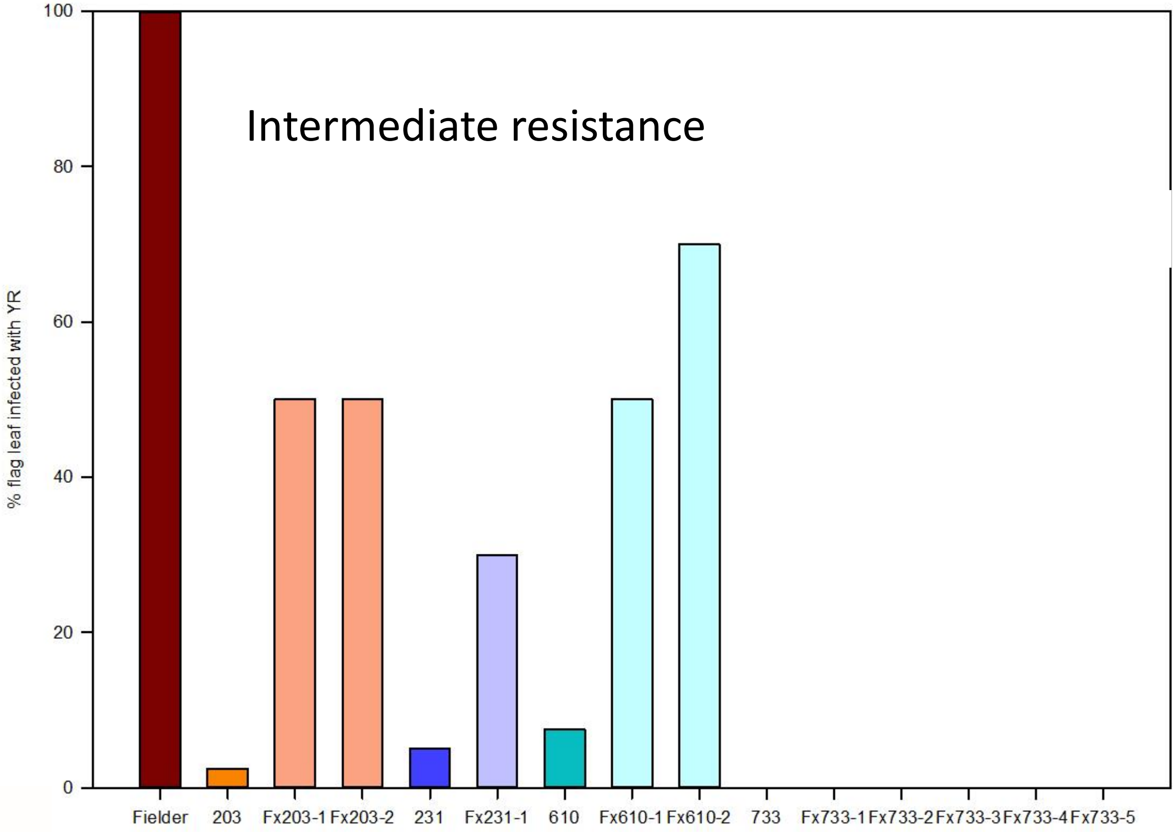

Department for Environment Food & Rural Affairs

F_1 plants – spring field trial

- Six F_1 grain from each of the 4 crosses sown
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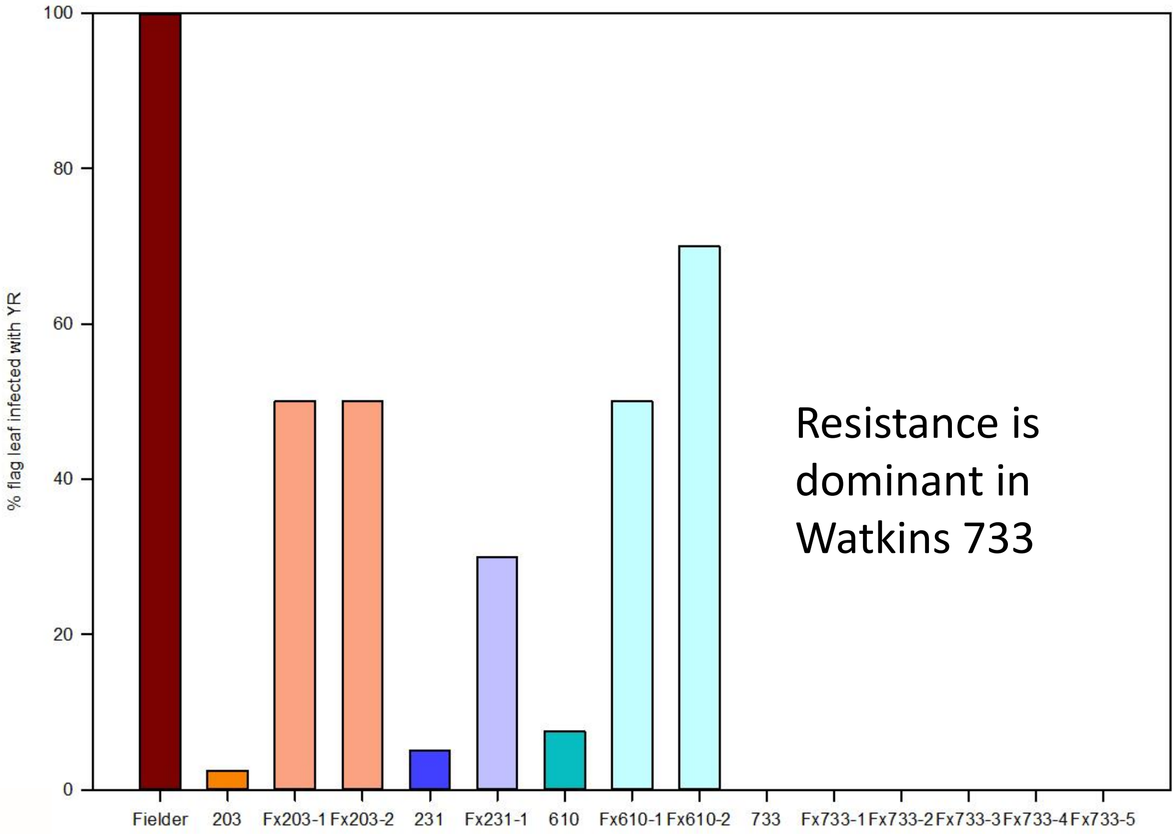


Resistance is dominant in Watkins 733

2017 F_2 field trial

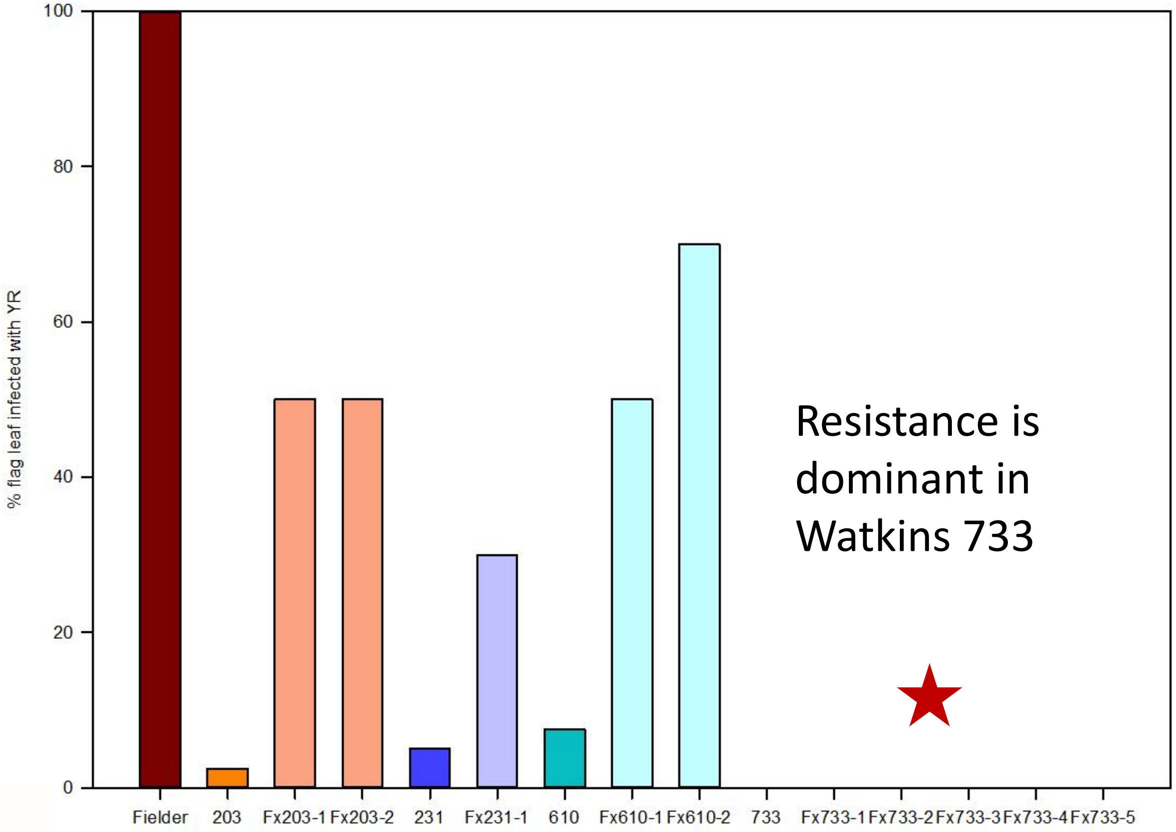
F_2 field trial

- Two or four F_2 populations from each of the four crosses sown (FxW203, FxW231, FxW610 and FxW733) + parents
- 10 F_1 plants sown from FxW786 cross
- Plot size = 4 rows x 1 m length, 60-80 seeds sown for each population
- Drilled 14th October 2016 in Sawyers 2
- After emergence and again in the spring plots were thinned to 40 plants per plot with approx. 10 cm spacing between plants

F_1 plants – spring field trial

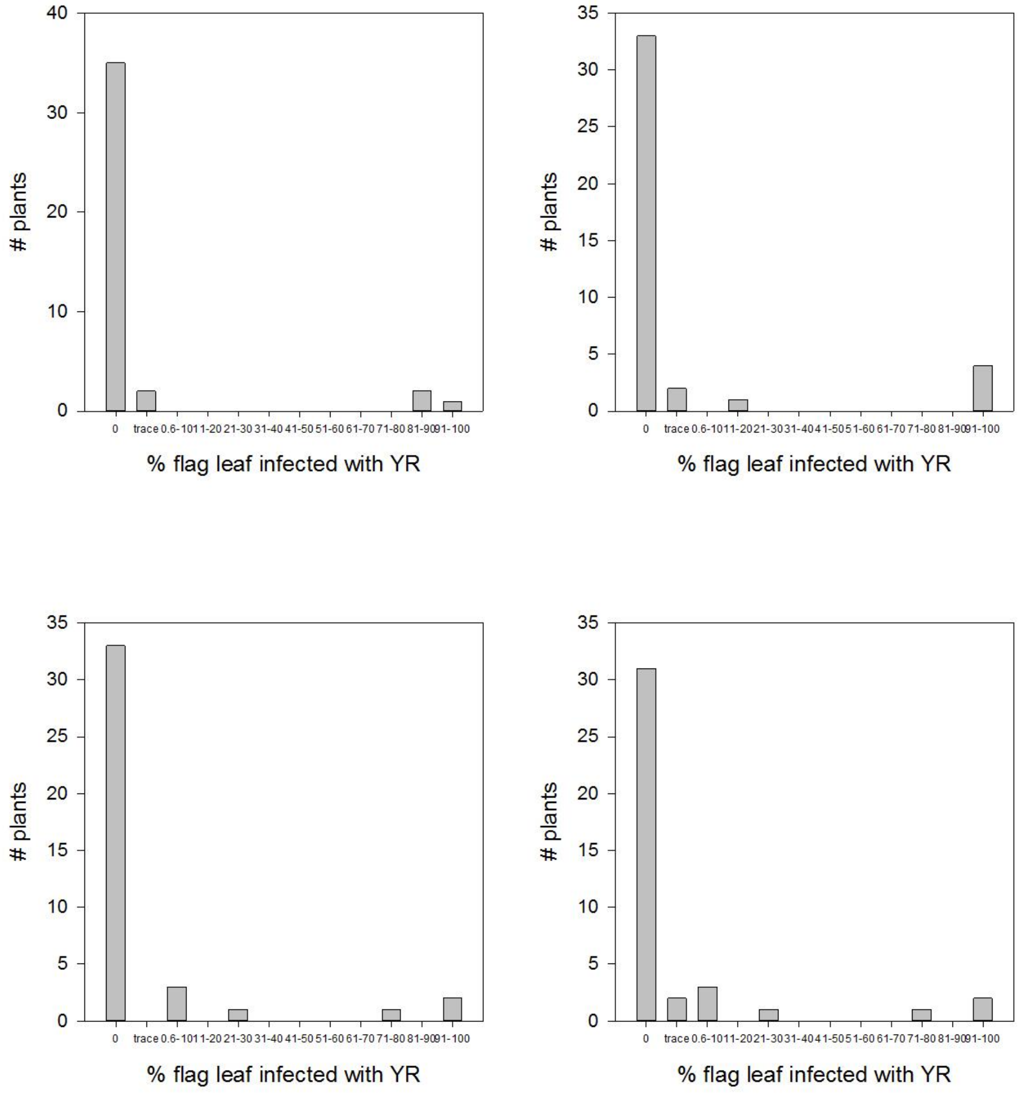
- Six F_1 grain from each of the 4 crosses sown
- Two replicate plots of parent genotypes (40 seeds per plot)

- Yellow Rust assessments on 30th June 2016



Resistance is dominant in Watkins 733

Fx733 F_2 segregation – flag leaf phenotyping



Complete resistance : Fully Susceptible
No sporulation : > 90% YR

132 R : 9 S
14.7 R : 1 S

Two unlinked dominant loci

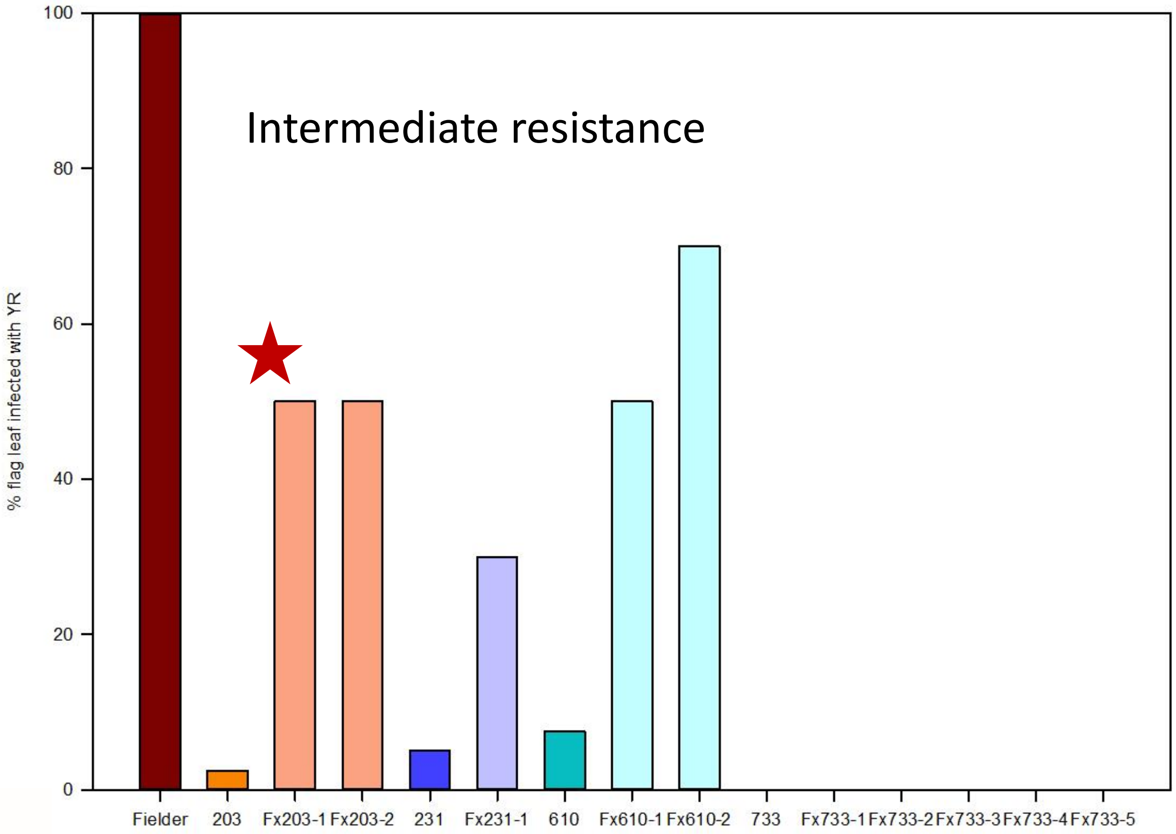
But some intermediate phenotypes:
Neighbouring plants with lots of inoculum
Other minor effect loci affecting overall plant physiology

Should give 3 R : 1 S ratio in testcross

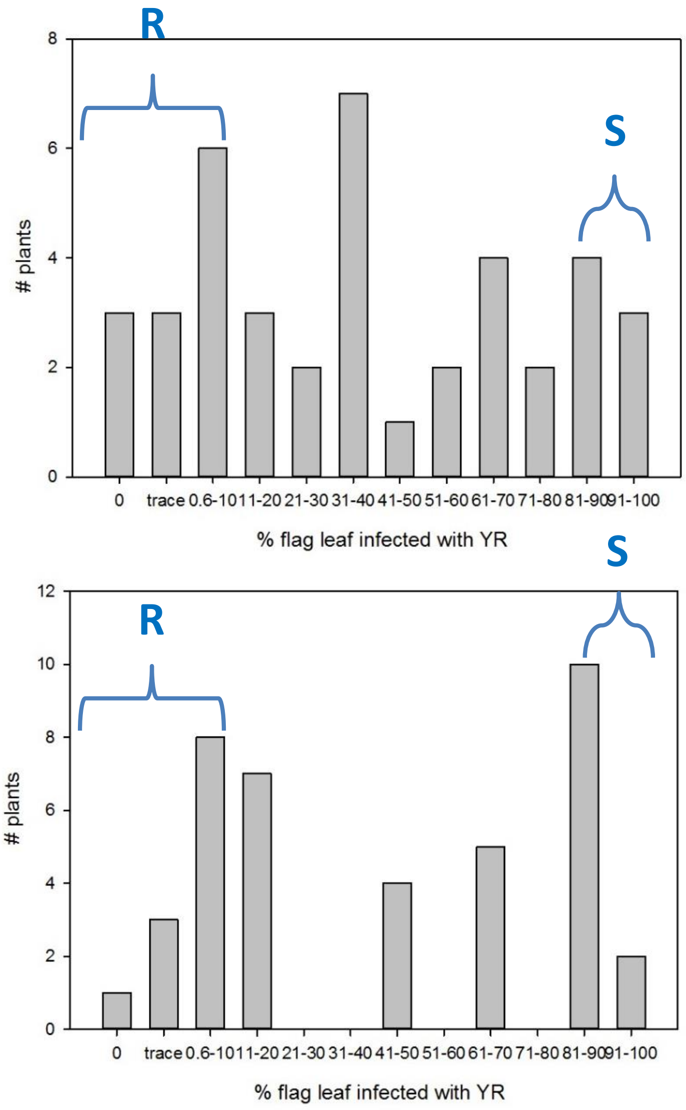
F_1 plants – spring field trial

- Six F_1 grain from each of the 4 crosses sown
- Two replicate plots of parent genotypes (40 seeds per plot)

- Yellow Rust assessments on 30th June 2016



Fx203 F_2 segregation – flag leaf assessments



24 R : 37 I : 19 S

1.2 R : 1.9 I : 1 S

Large number of intermediates

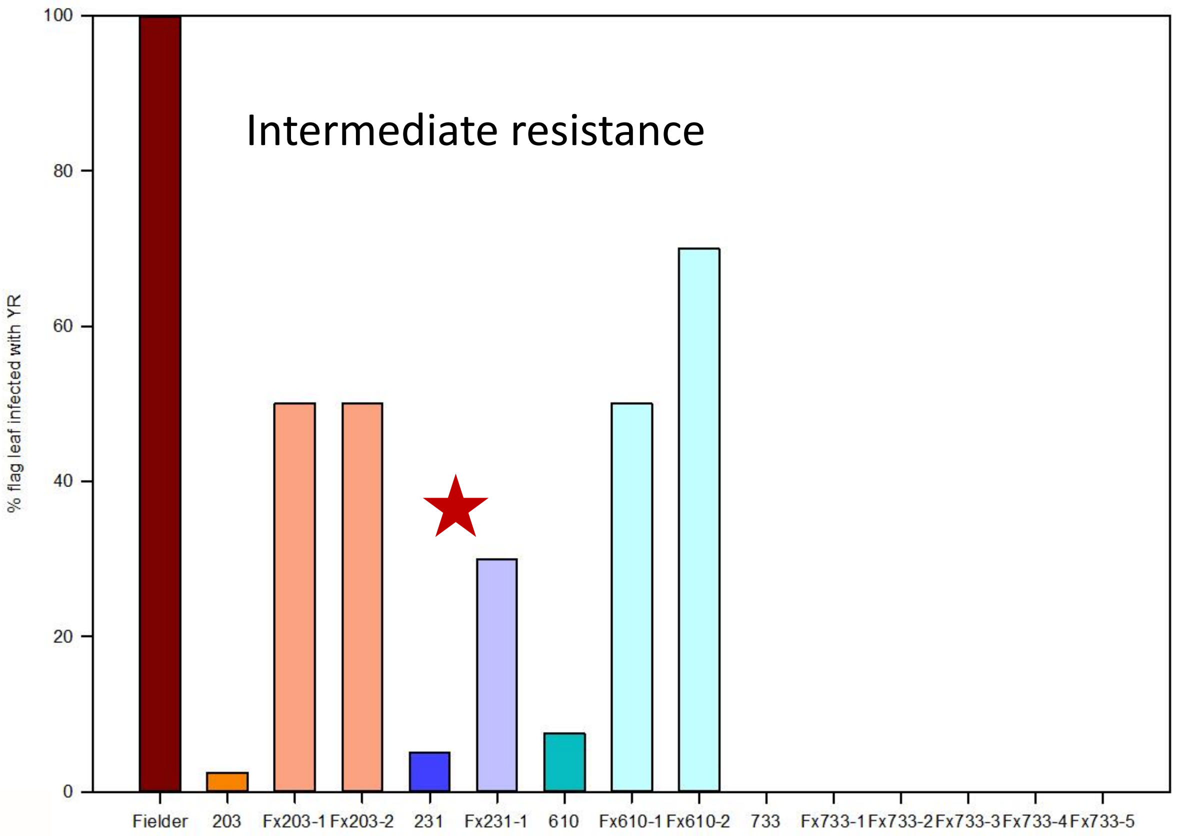
Semi-dominant resistance locus

Should give 1 I : 1 S ratio in testcross

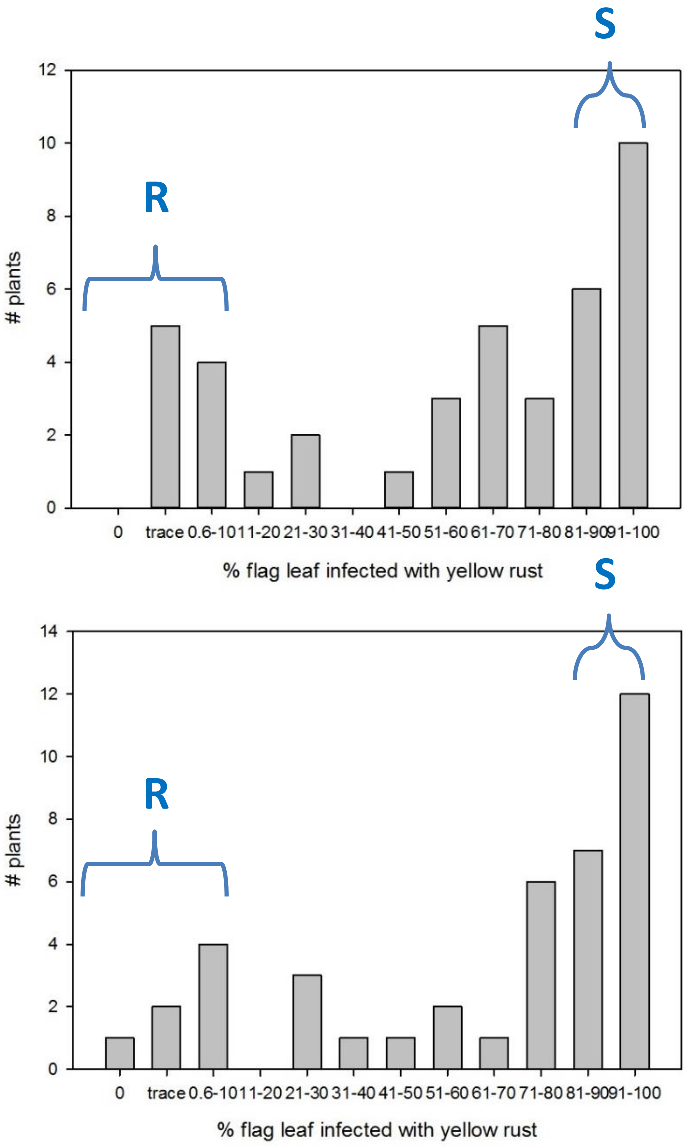
F_1 plants – spring field trial

- Six F_1 grain from each of the 4 crosses sown
- Two replicate plots of parent genotypes (40 seeds per plot)

- Yellow Rust assessments on 30th June 2016



Fx231 F_2 segregation – flag leaf assessments



16 R : 29 I : 35 S

1 : 2 : 2

Weakest parental resistance

Semi-dominant resistance in W231

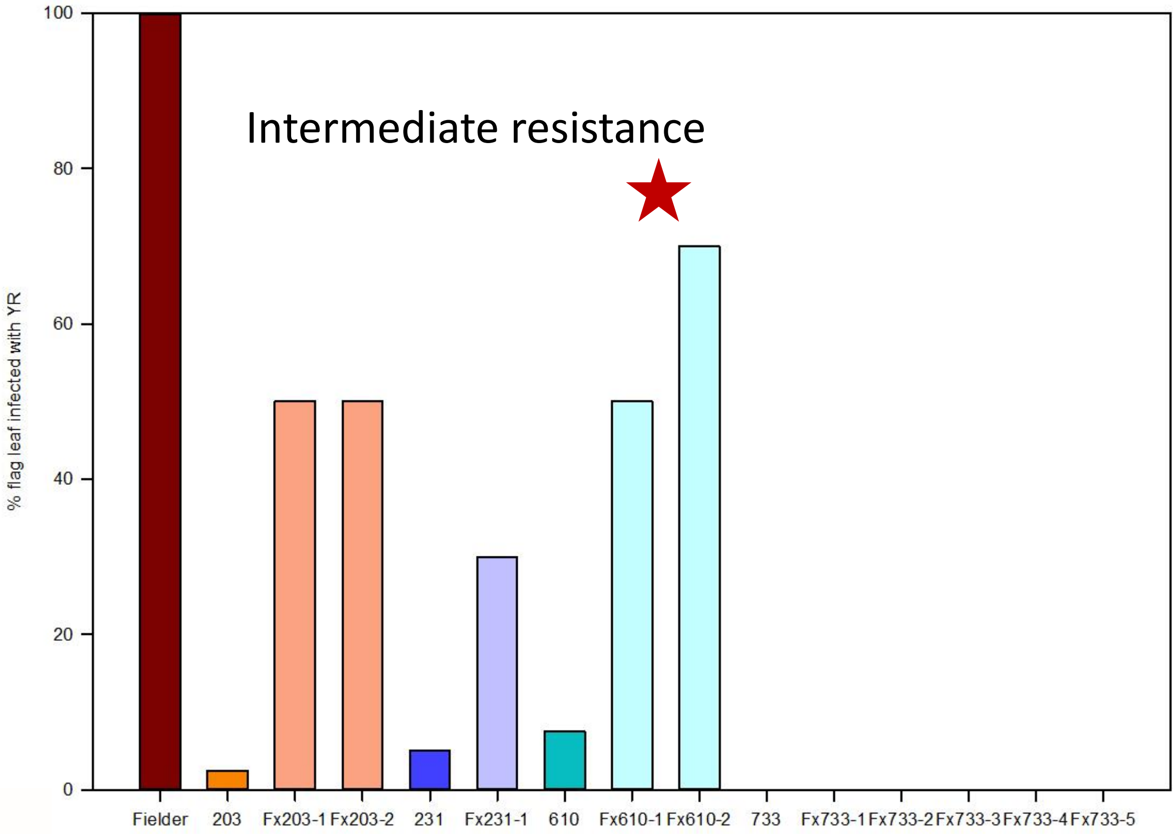
Skewed towards fully susceptible phenotype

Suppressor locus from Fielder?

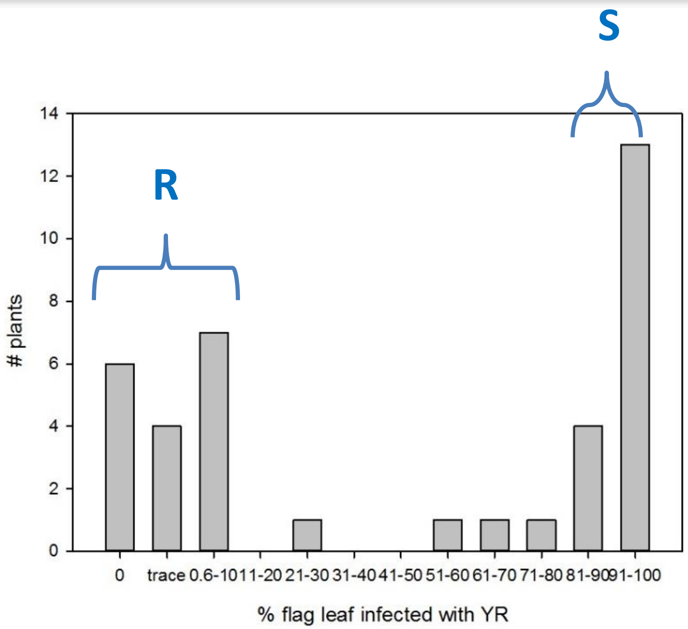
F_1 plants – spring field trial

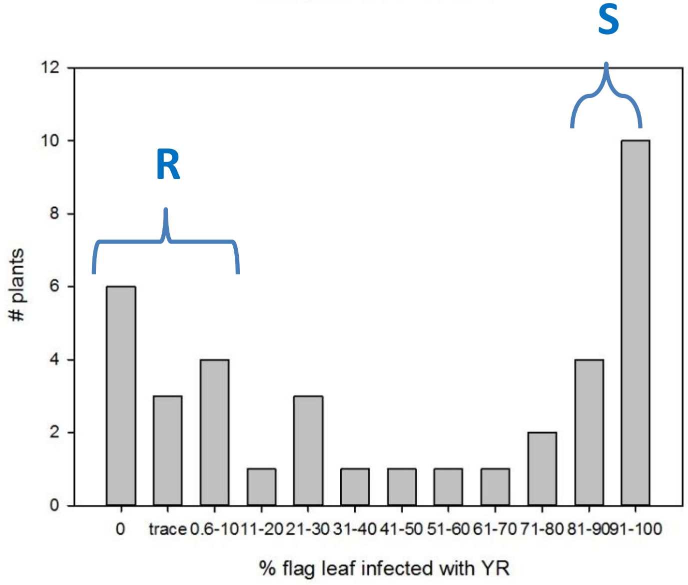
- Six F_1 grain from each of the 4 crosses sown
- Two replicate plots of parent genotypes (40 seeds per plot)

- Yellow Rust assessments on 30th June 2016



Fx610 F_2 segregation – flag leaf assessments





30 R : 14 I : 32 S

2 : 1 : 2

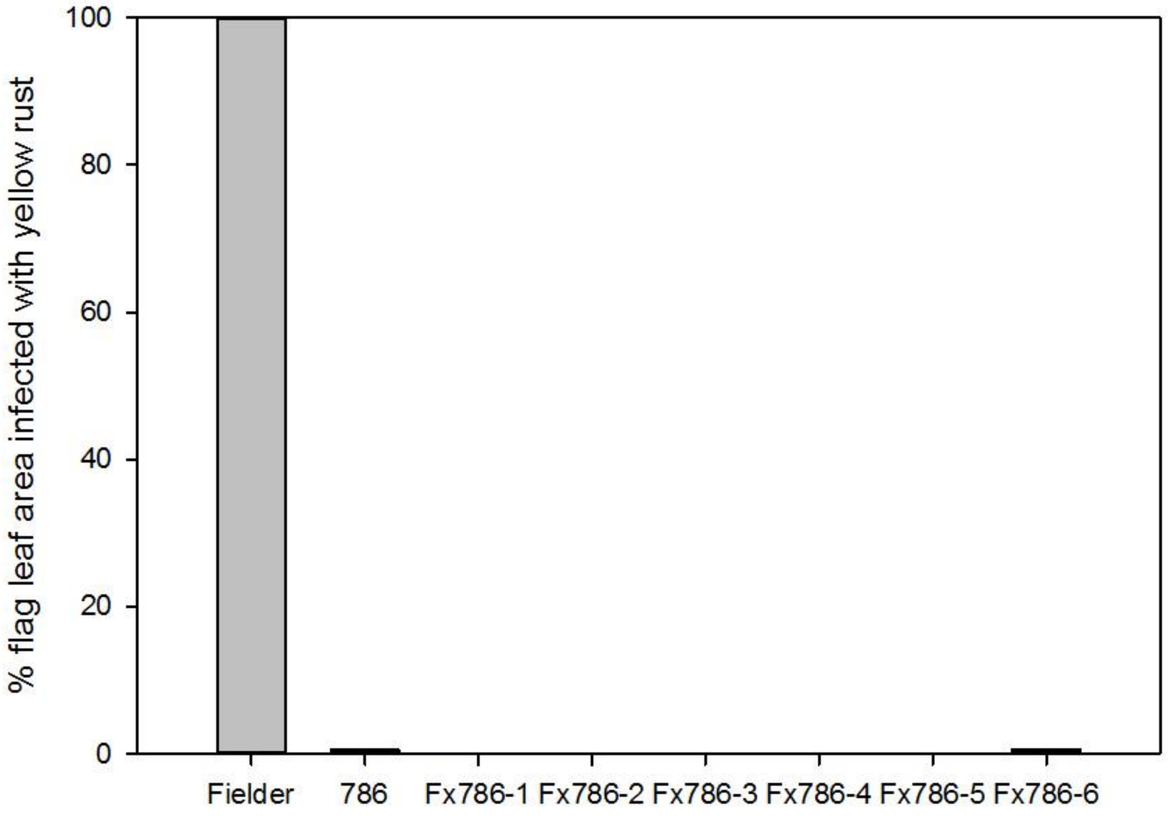
Semi-dominant resistance locus

Lack of intermediate phenotypes

Interaction effect with other loci?

610 physiological later maturing variety

Fx786 F_1 phenotype - flag leaf assessments



Resistance is dominant in Watkins 786

Next steps

Sow testcross trial in field (F_1 backcrossed to Fielder)

backcross	**harvested**
203	**145**
231	**169**
610	**171**
733	**175**

Include F_1 plants and parental genotypes to benchmark the phenotype of known hets – confirm if we see variation in hets due to potential suppressor alleles

Sow F_2 FxW786 populations

Fx786 F_1 Plant	F_2 harvested seed
1	579
2	610
3	605
4	251
5	No seed
6	335

Summary

- Five Watkins accessions with evidence of moderate-high resistance against YR across three field seasons (and 2008 field trial)

- Watkins 203 and 610 most promising for multi-disease resistance (although 610 later maturing)

- Genetics of inheritance different in each Watkins accession

(no single dominant locus 3R : 1S ratios)

2017-2018 field trials
Testcross trial
MDR031 (R) x MDR043 (S) take-all phenotyping trial
Watkins lines also included in Zymoseptoria field trial

Many thanks to

Kim Hammond-Kosack

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

Gail Canning - seed preparation

Mike-Hammond-Kosack - crossing

Rodger White - statistics

RRes farm and glasshouse staff

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for Environment
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Wheat
Genetic
Improvement
Network

WGIN Wheat Promotome Capture

A sub-contractor project of WGIN 3

Michael Hammond-Kosack (WGIN PA)

Update for WGIN MM on October 6th @ RRes

ROTHAMSTED
RESEARCH

What is Promotome Capture & what are the **Main Objectives** for WGIN ?

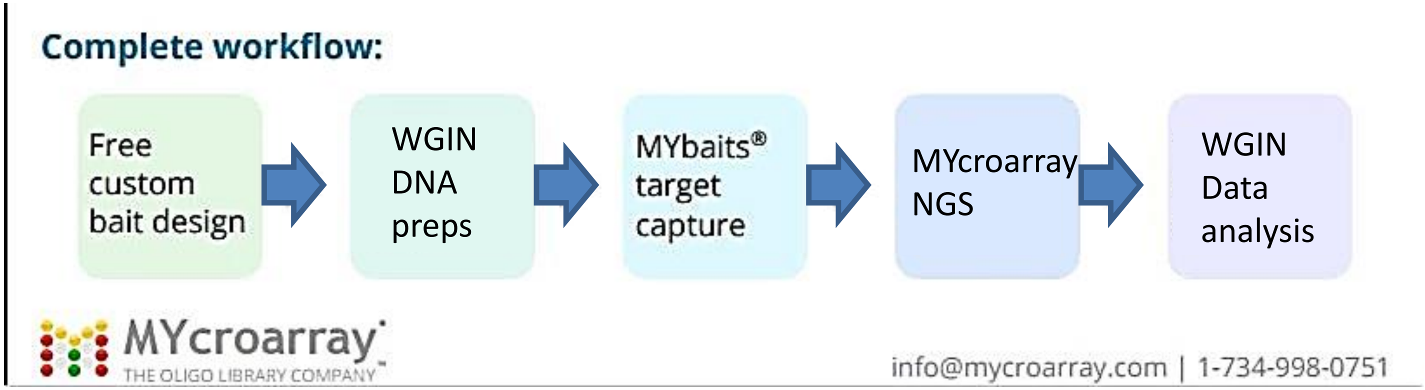
What Is It?

~~Exome~~ Promotome Capture

“~~Exome~~ Promotome capture is a method used to extract and sequence the ~~exome~~ promotome (collection of all ~~exons~~ promoters) in a genome and compare this variation across a sample of individual organisms (wheat cultivars). This allows studies to quickly focus in on the small percent of the genome that is most likely to contain variation that strongly affects phenotypes of interest and/or to identify rates of ~~codon~~ promoter evolution between a set of species to infer the effects of mutation and selection among genes.” (http://hawaiireedlab.com/gwiki/index.php?title=Exome_Capture)

Main Objectives

- Comparison of promoters of genes of interest in a large number of wheat cultivars used in breeding, farming & scientific research
- Identification of cis-acting elements important for gene regulation
- Linking this comparison to phenotypic and transcriptomics data to increase our understanding of gene regulation to generate these phenotypes



Complete workflow:

Free custom bait design → WGIN DNA preps → MYbaits® target capture → MYcroarray NGS → WGIN Data analysis

MYcroarray®
THE OLIGO LIBRARY COMPANY™

info@mycroarray.com | 1-734-998-0751

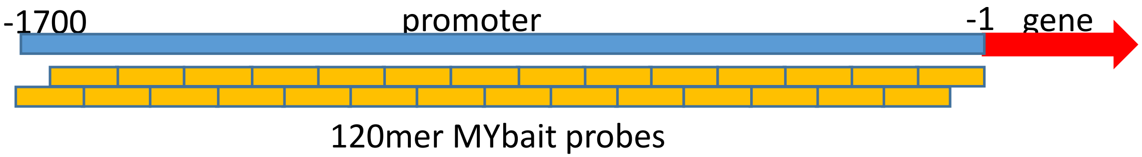
Time Line

1. FASTA with 1,402 promoter sequences submitted to MYcroarray **May 10th**
2. MYbaits synthesis (@ Mycroarray) completed **August 24th**
3. 96 DNA samples (80%, up to 8µg) posted **September 11th**
4. Samples received by MYcroarray **September 12th**
5. MYbaits Capture: Library preps started **September 14th**
6. Next Generation Sequencing @ MYcroarray: **October**
7. Data will be received @ RRes from **mid November 2017**
8. Bio-Informatics **November 2017 – February 2050** (WGIN extension 18)

WGIN Funding & Design - Traits, Trait-Coordinators & Wheat Cultivars

- Defra’s WGIN 3 funding for this sub-contractor project allowed for the use of up to **40,000 MYbait probes** and **96 “Reactions”** (which equates to **96 Wheat Cultivars**)

- **MYbaits set-up**: **1700bp promoter** uses **28** MYbaits (120bp) with **2 fold** coverage :



- 3 wheat homoeologues (A, B, D) **3 x 28 MYbait probes / gene**

- Thus Defra’s funding will allow for the capture of **1428 promoter sequences**.

- The WGIN Management Team decided on **10 Trait Categories**,
  and thus ≤**143 promoter sequences per trait**

Traits & Trait-Coordinators

1. Yield Resilience – Cristobal Uauy (JIC)

2. Grain Composition – Peter Shewry, Rowan Mitchell (RRes), Kay Trafford (NIAB)

3. Grain Development – Cristobal Uauy (JIC)

4. Biotic Stress (fungi and insects) – Matthew Moscou (TSL), Kim Hammond-Kosack (RRes)

5. Abiotic Stress (drought, high temp) – John Foulkes (UoN)

6. Nutrient Use Efficiency – Malcolm Hawkesford (RRes)

7. Canopy Development / Whole Plant Architecture – Clare Lister & Simon Griffiths (JIC)

8. Flower biology – Zoe Wilson (UoN), Scott Bowden (JIC)

9. Root architecture – Malcolm Bennett (UoN), Peter Buchner (RRes)

10. Recombination – James Higgins (Leicester Uni)

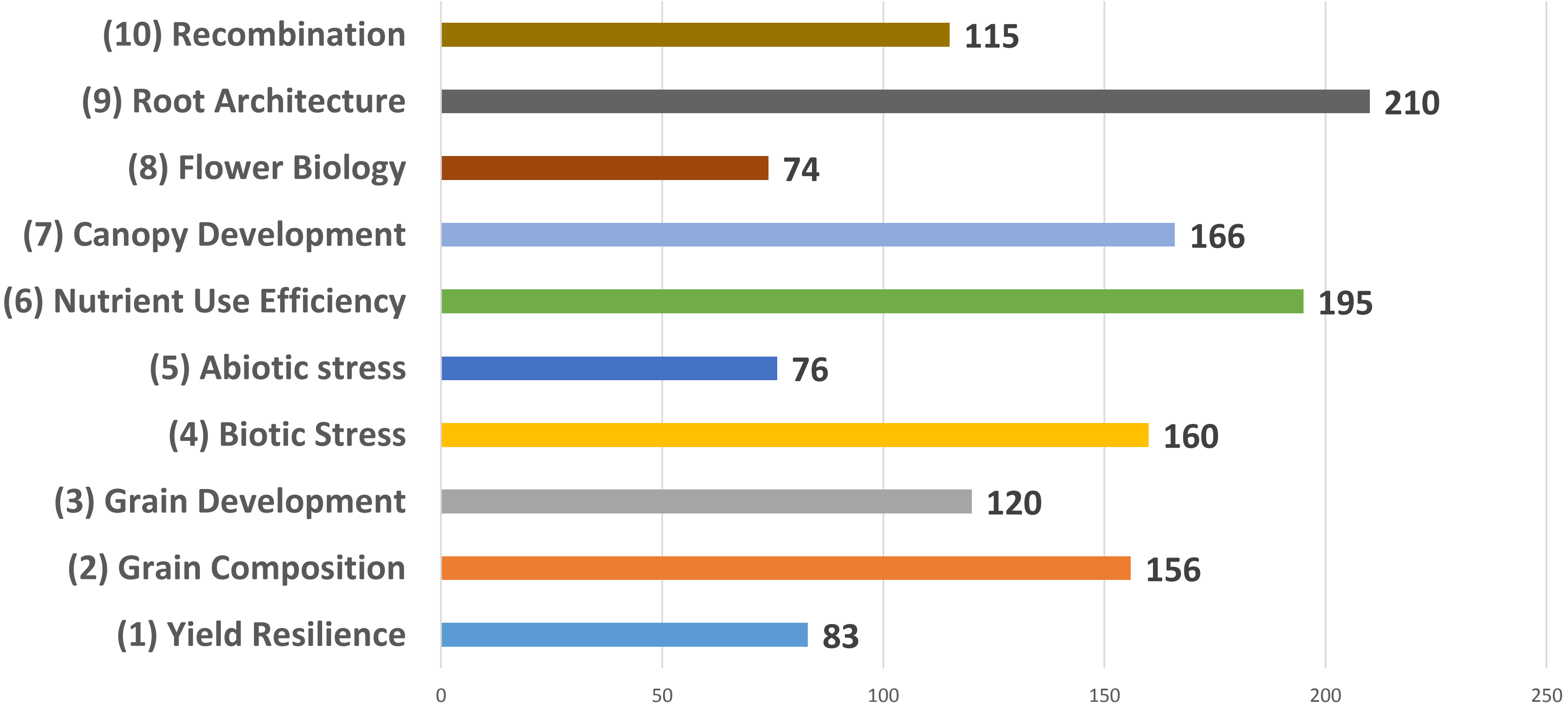
The 96 Wheat Cultivars

Chosen by WGIN Management Team Members and Trait Coordinators

ID	cultivar	nominators
1	A. speltoides 2140022	LS/GA
2	Abbot	SB
3	Aegilops peregrina (variabilis)	KT
4	Alcedo	RAGT
5	Ambrosia	SB
6	Avalon	SG/MH/VM/SB/CL/EO+JF
7	Badger	CL
8	Bobwhite	KK
9	Brompton	LG
10	Buster	SB
11	Cadenza	SG/MH/VM/SB/CL
12	Cellule	RAGT
13	Charger	SB
14	Chinese Spring	SG
15	Claire	RAGT/SG/PS/MH/CL
16	Coppadra	KK
17	Cordiale	PS/MH
18	Cougar	RAGT
19	Crusoe	RAGT/MH
20	Dickens	LG
21	Einstein	SB
22	ENT-228	LS/GA
23	Fielder	KK
24	Flanders	RAGT
25	Gallant	MH
26	Garcia	SG/CL
27	Gatsby	ECS
28	Gladiator	SB
29	Graham	LG/syn
30	Hereford	KHK/VM
31	Hereward	RAGT/PS/MH/SB/CL
32	Hobbit	SB
33	Hustler	SB
34	Isengrain	PS
35	Istabraq	PS/MH
36	JB Diego	RAGT
37	Kronos	WGIN MM team
38	KWS Santiago	RAGT
39	KWS Silverstone	LG
40	KWS Siskin	RAGT
41	KWS Trinity	LG
42	Malacca	PS/MH/CL
43	Maris Huntsman	SB
44	Maris Widgeon	MH
45	Marksman	PS
46	Mercia	MH
47	Napier	CL
48	Oakley	RAGT
49	Paragon	SG/MH/AR/VM
50	Piko	RAGT
51	Reflection	LG
52	Relay	RAGT
53	Revelation	LG
54	Rialto	RAGT/SB/PS
55	Riband	MH/KK
56	Robigus	RAGT/SG/MH/AR
57	Savannah	CL
58	Scout	RAGT
59	Sear Synthetic	SG
60	Skyfall	RAGT
61	Soisson	RAGT/PS/MH
62	Solstice	RAGT/GA_LS/MH
63	Spark	PS/CL
64	Stigg	MH
65	Sumai 3	RAGT
66	*T. monoccocum* MDR031	KHK/VM
67	*T. monoccocum* MDR037	LS/GA/VM
68	*T. monoccocum* MDR043	KHK/VM
69	*T. monoccocum* MDR045	LS/GA
70	*T. monoccocum* MDR046	RRES/VM
71	*T. monoccocum* MDR049	LS/GA
72	*T. monoccocum* MDR308	KHK
73	*T. monoccocum* MDR657	LS/GA
74	Taichung 29	KK/JRudd
75	Ukrainka	PS
76	USU-Apogee	KK
77	Valoris	PS
78	Veranopolis	KK
79	Watkins 115	LS/GA
80	Watkins 141	SG
81	Watkins 160	SG
82	Watkins 199	LS/GA/SG
83	Watkins 203	VM
84	Watkins 239	SG
85	Watkins 209	
86	Watkins 246	SG
87	Watkins 292	SG
88	Watkins 387	SG
89	Watkins 579	LS/GA
90	Watkins 624	LS/GA
91	Watkins 733	VM
92	Watkins 777	VM/SG
93	Watkins 786	VM
94	Xi19	RAGT/PS/MH
95	Yumai 34	PS
96	Zebedee	EO+JF

Gene IDs received & Workflow

A total of **1355 Gene IDs** distributed as:



Workflow

1

- Retrieve coding sequences (CDS) from **Ensembl Biomart** (http://plants.ensembl.org/biomart)

2

- BLAST CDS against **IWGSC RefSeq v1.0** (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies):
- Obtain coordinates on chromosome with 100% ID

3

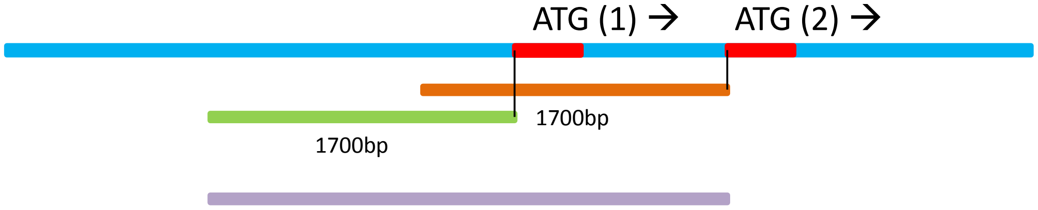
- Use coordinates on relevant RefSeq v1.0 chromosome in Geneious to obtain 1700bp upstream of ATG
- Generate FASTA file with all promoter/5’UTR seqs

Too Many Promoters!

MYbaits 2 set (max. 40,000 baits):

- **88 genes** have (up to 4) **alternate ATGs**
- Inclusion of 1700bp upstream for all of these exceeded the max MYbaits number
- Including promoters for **ALL genes and ALL alternate start sites** needed a **reduction of 1100 MYbaits**: achieved by combining overlapping promoters

a) overlap: combined promoter with shared MYbaits (< <56 MYbaits/promoter)



ATG (1) →

ATG (2) →

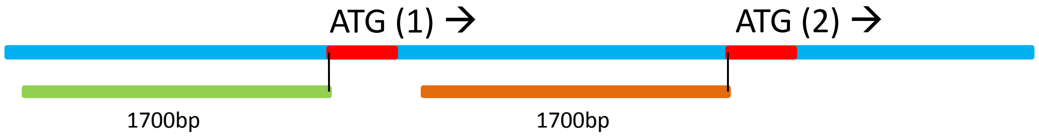
1700bp

1700bp

Combined promoter: >1700bp and <3400bp

1A-0075430-T6-9 (2717bp)

b) **NO** overlap: 2 separate promoters with individual MYbaits (56 MYbaits/promoter)



ATG (1) →

ATG (2) →

1700bp

1700bp

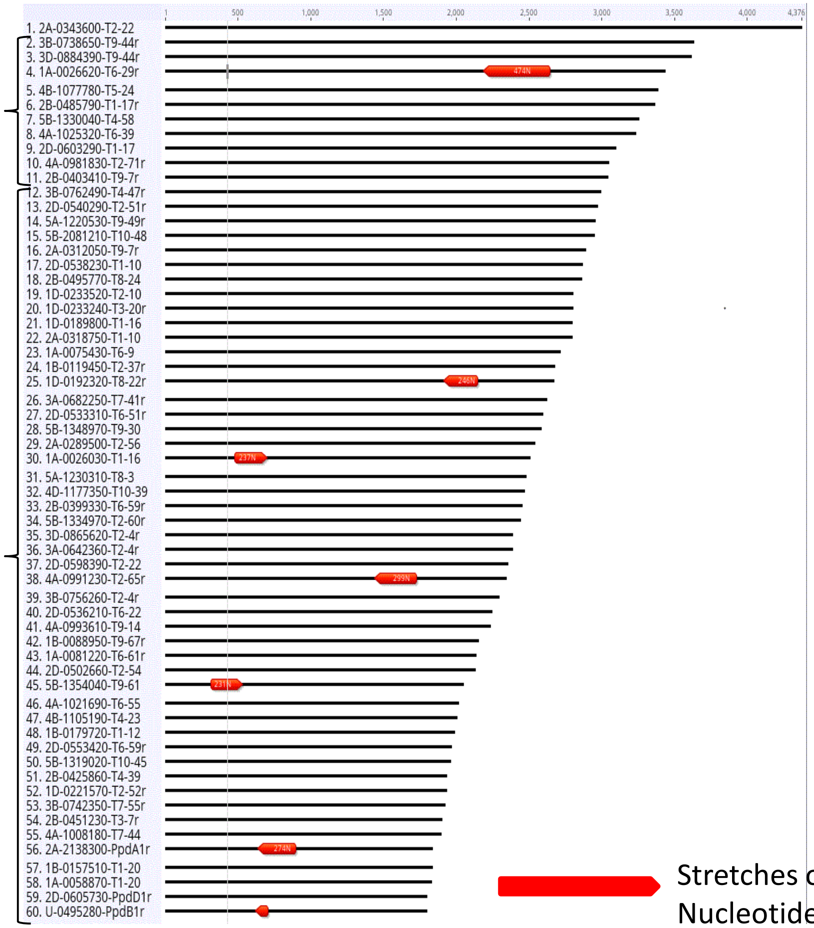
1A-0017770-T9-11-1

1A-0017770-T9-11-2

Promoters for 60 genes combined

saving 1,157 MyBaits

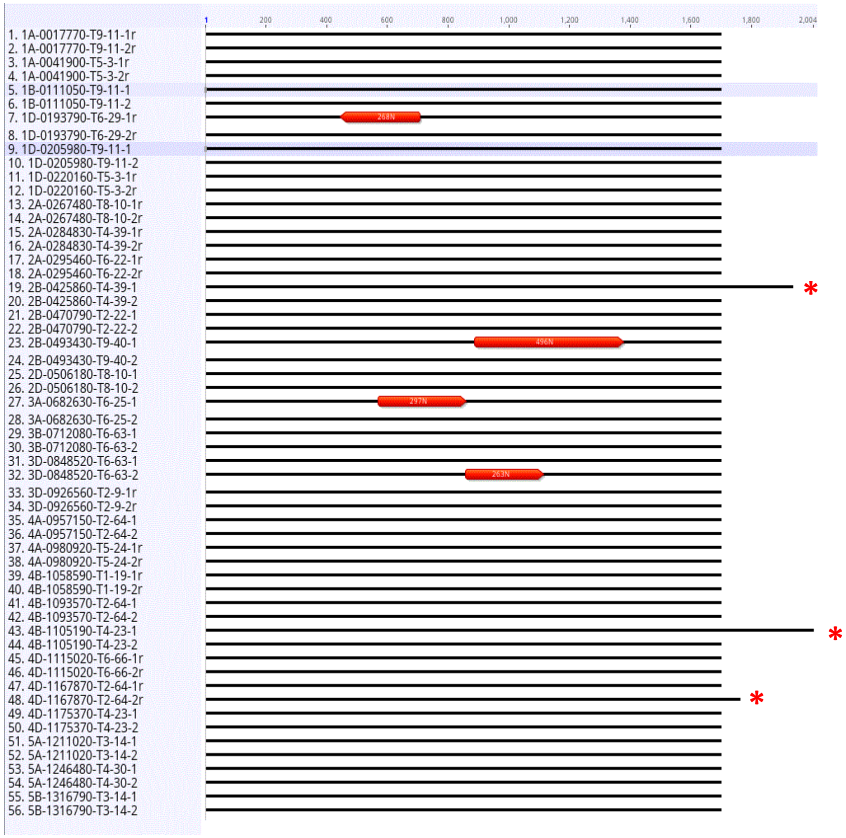
Promoters combined: 4



1. 2A-0343600-T2-22
2. 3B-0738650-T9-44r
3. 3D-0884390-T9-44r
4. 1A-0026620-T6-29r
5. 4B-1077780-T5-24
6. 2B-0485790-T1-17r
7. 5B-1330040-T4-58
8. 4A-1025320-T6-39
9. 2D-0603290-T1-17
10. 4A-0981830-T2-71r
11. 2B-0403410-T9-7r
12. 3B-0762490-T4-47r
13. 2D-0540290-T2-51r
14. 5A-1220530-T9-49r
15. 5B-2081210-T10-48
16. 2A-0312050-T9-7r
17. 2D-0538230-T1-10
18. 2B-0495770-T8-24
19. 1D-0233520-T2-10
20. 1D-0233240-T3-20r
21. 1D-0189800-T1-16
22. 2A-0318750-T1-10
23. 1A-0075430-T6-9
24. 1B-0119450-T2-37r
25. 1D-0192320-T8-22r
26. 3A-0682250-T7-41r
27. 2D-0533310-T6-51r
28. 5B-1348970-T9-30
29. 2A-0289500-T2-56
30. 1A-0026030-T1-16
31. 5A-1230310-T8-3
32. 4D-1177350-T10-39
33. 2B-0399330-T6-59r
34. 5B-1334970-T2-60r
35. 3D-0865620-T2-4r
36. 3A-0642360-T2-4r
37. 2D-0598390-T2-22
38. 4A-0991230-T2-65r
39. 3B-0756260-T2-4r
40. 2D-0536210-T6-22
41. 4A-0993610-T9-14
42. 1B-0088950-T9-67r
43. 1A-0081220-T6-61r
44. 2D-0502660-T2-54
45. 5B-1354040-T9-61
46. 4A-1021690-T6-55
47. 4B-1105190-T4-23
48. 1B-0179720-T1-12
49. 2D-0553420-T6-59r
50. 5B-1319020-T10-45
51. 2B-0425860-T4-39
52. 1D-0221570-T2-52r
53. 3B-0742350-T7-55r
54. 2B-0451230-T3-7r
55. 4A-1008180-T7-44
56. 2A-2138300-PpdA1r
57. 1B-0157510-T1-20
58. 1A-0058870-T1-20
59. 2D-0605730-PpdD1r
60. U-0495280-PpdB1r

🟥 Stretches of non-determinate Nucleotides (Ns)

28 Promoters not (fully*) combined



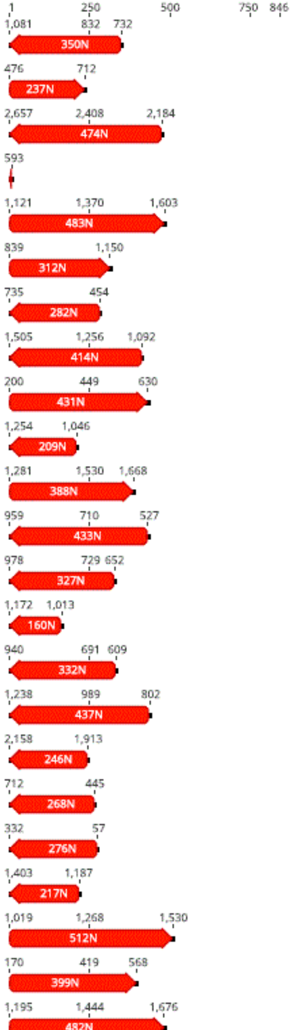
1. 1A-0017770-T9-11-1r
2. 1A-0017770-T9-11-2r
3. 1A-0041900-T5-3-1r
4. 1A-0041900-T5-3-2r
5. 1B-0111050-T9-11-1
6. 1B-0111050-T9-11-2
7. 1D-0193790-T6-29-1r
8. 1D-0193790-T6-29-2r
9. 1D-0205980-T9-11-1
10. 1D-0205980-T9-11-2
11. 1D-0220160-T5-3-1r
12. 1D-0220160-T5-3-2r
13. 2A-0267480-T8-10-1r
14. 2A-0267480-T8-10-2r
15. 2A-0284830-T4-39-1r
16. 2A-0284830-T4-39-2r
17. 2A-0295460-T6-22-1r
18. 2A-0295460-T6-22-2r
19. 2B-0425860-T4-39-1
20. 2B-0425860-T4-39-2
21. 2B-0470790-T2-22-1
22. 2B-0470790-T2-22-2
23. 2B-0493430-T9-40-1
24. 2B-0493430-T9-40-2
25. 2D-0506180-T8-10-1
26. 2D-0506180-T8-10-2
27. 3A-0682630-T6-25-1
28. 3A-0682630-T6-25-2
29. 3B-0712080-T6-63-1
30. 3B-0712080-T6-63-2
31. 3D-0848520-T6-63-1
32. 3D-0848520-T6-63-2
33. 3D-0926560-T2-9-1r
34. 3D-0926560-T2-9-2r
35. 4A-0957150-T2-64-1
36. 4A-0957150-T2-64-2
37. 4A-0980920-T5-24-1r
38. 4A-0980920-T5-24-2r
39. 4B-1058590-T1-19-1r
40. 4B-1058590-T1-19-2r
41. 4B-1093570-T2-64-1
42. 4B-1093570-T2-64-2
43. 4B-1105190-T4-23-1
44. 4B-1105190-T4-23-2
45. 4D-1115020-T6-66-1r
46. 4D-1115020-T6-66-2r
47. 4D-1167870-T2-64-1r
48. 4D-1167870-T2-64-2r
49. 4D-1175370-T4-23-1
50. 4D-1175370-T4-23-2
51. 5A-1211020-T3-14-1
52. 5A-1211020-T3-14-2
53. 5A-1246480-T4-30-1
54. 5A-1246480-T4-30-2
55. 5B-1316790-T3-14-1
56. 5B-1316790-T3-14-2

* For these 3 genes, 2 out of 3 alternate Promoters were combined

Promoters/UTRs with Undeterminate Nucleotide Stretches (Ns)

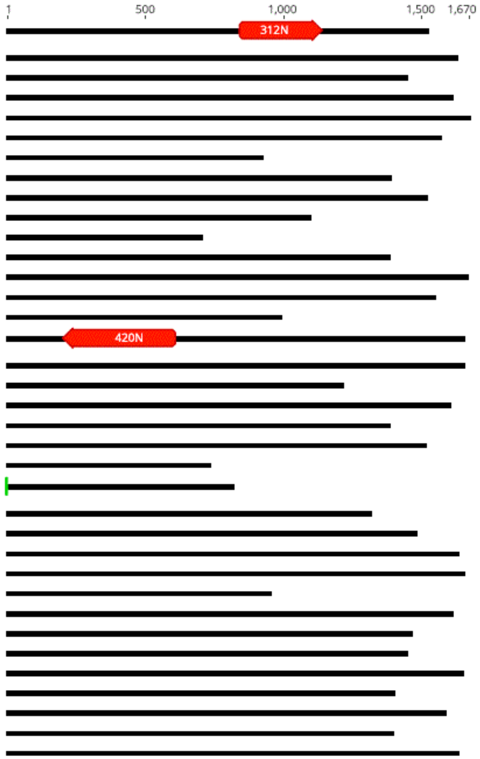
a) N stretches **within** 1700bp: 136

1. 1A-0024490-T8-11r - 350N
2. 1A-0026030-T1-16 - 237N
3. 1A-0026620-T6-29r - 474N
4. 1A-0031460-T4-49r - 10N
5. 1A-0036950-T10-23 - 483N
6. 1A-0037970-T7-20 - 312N
7. 1A-0047200-T3-20r - 282N
8. 1A-0069860-T4-51r - 414N
9. 1A-0075220-T2-50 - 431N
10. 1B-0088580-T1-6r - 209N
11. 1B-0108420-T2-52 - 388N
12. 1B-0108930-T6-29r - 433N
13. 1B-0122280-T4-40r - 327N
14. 1B-0123110-T8-22r - 160N
15. 1B-0148870-T4-49r - 332N
16. 1B-0150920-T6-48r - 437N
17. 1D-0192320-T8-22r - 246N
18. 1D-0193790-T6-29-1r - 268N
19. 1D-0221450-T7-42r - 276N
20. 1D-0221450-T7-42r - 217N
21. 1D-0258100-T1-25 - 512N
22. 1D-0264750-T6-48 - 399N
23. 2A-0323970B-T1-5 - 482N

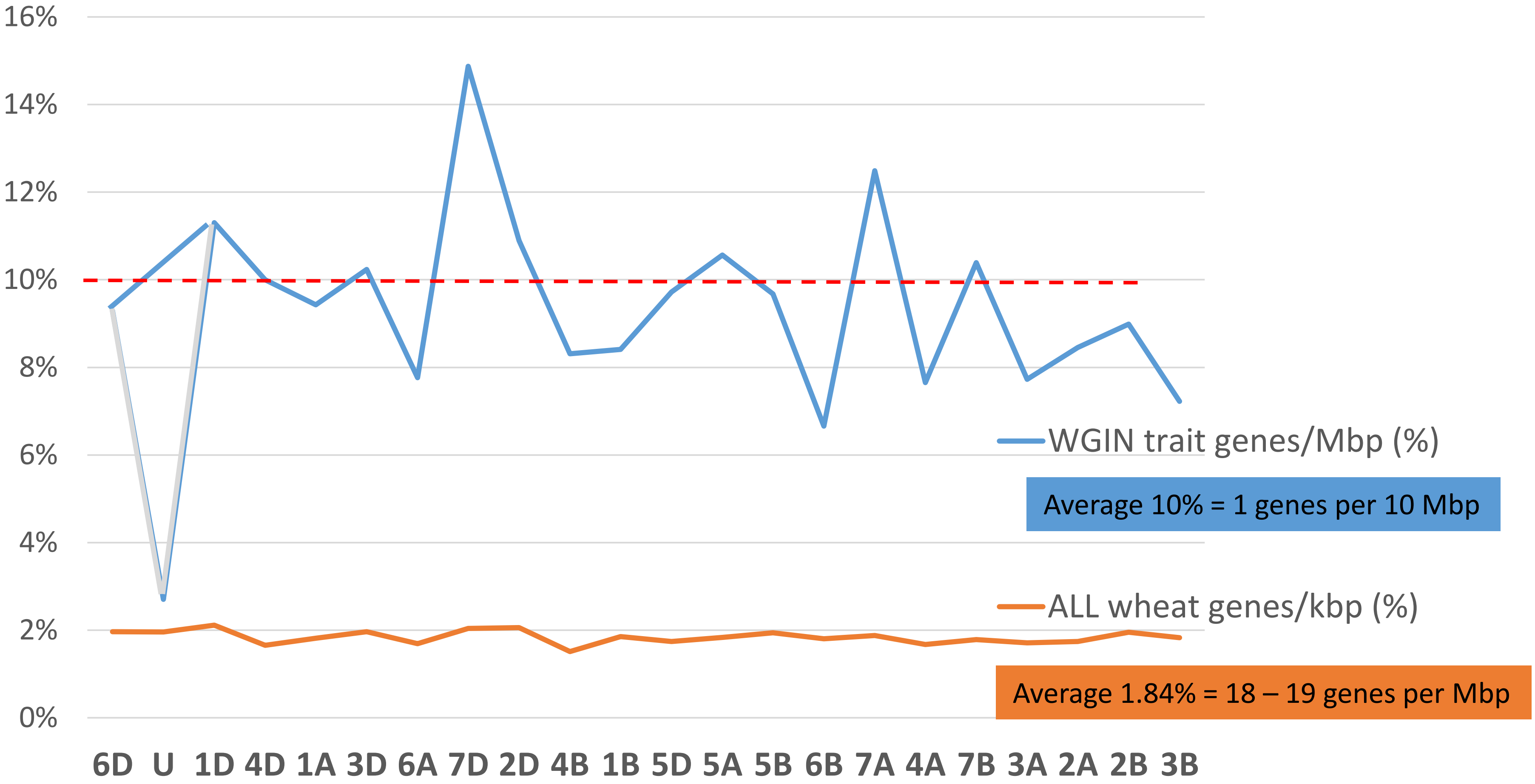


b) N stretches **reducing** 1700bp: 36

1. 1A-0037970-T7-20
2. 1A-0080120-T5-30r
3. 1B-0103850-T7-3r
4. 1B-0156080-T5-12
5. 2A-0343000-T2-31r
6. 2B-0425980-T2-56r
7. 3A-0686640-T7-2
8. 3B-0725290-T7-21r
9. 3D-0898770-T7-21r
10. 3D-0913830-T9-37
11. 3D-0926370-T5-26
12. 3D-0928740-T7-24
13. 3D-0935160-T2-28r
14. 4A-1016270-T9-19
15. 4A-1023210-T8-5r
16. 4B-1041080-T4-24r
17. 4B-1052320-T5-10r
18. 4B-1109570-T2-73
19. 5A-1186440-T4-36
20. 5A-1192740-T1-3
21. 5A-1216860-T1-23r
22. 5A-1219370-T3-10r
23. 5A-1252880-T9-29r
24. 5B-1310950-T6-24
25. 5B-1313490-T1-23r
26. 5B-1323430-T1-3r
27. 5D-1434480-T5-16B
28. 5D-1448500-T9-8
29. 6A-1566480-T8-7r
30. 6B-1587290-T9-18
31. 6B-1648900-T6-15r
32. 7A-1759120-T6-33
33. 7A-1831290-T4-54r
34. 7B-2140600B-T4-32r
35. 7D-1974260-T6-3r
36. 7D-2008610-T9-15r



Distribution of WGIN Trait Genes in Wheat Genome is NOT even



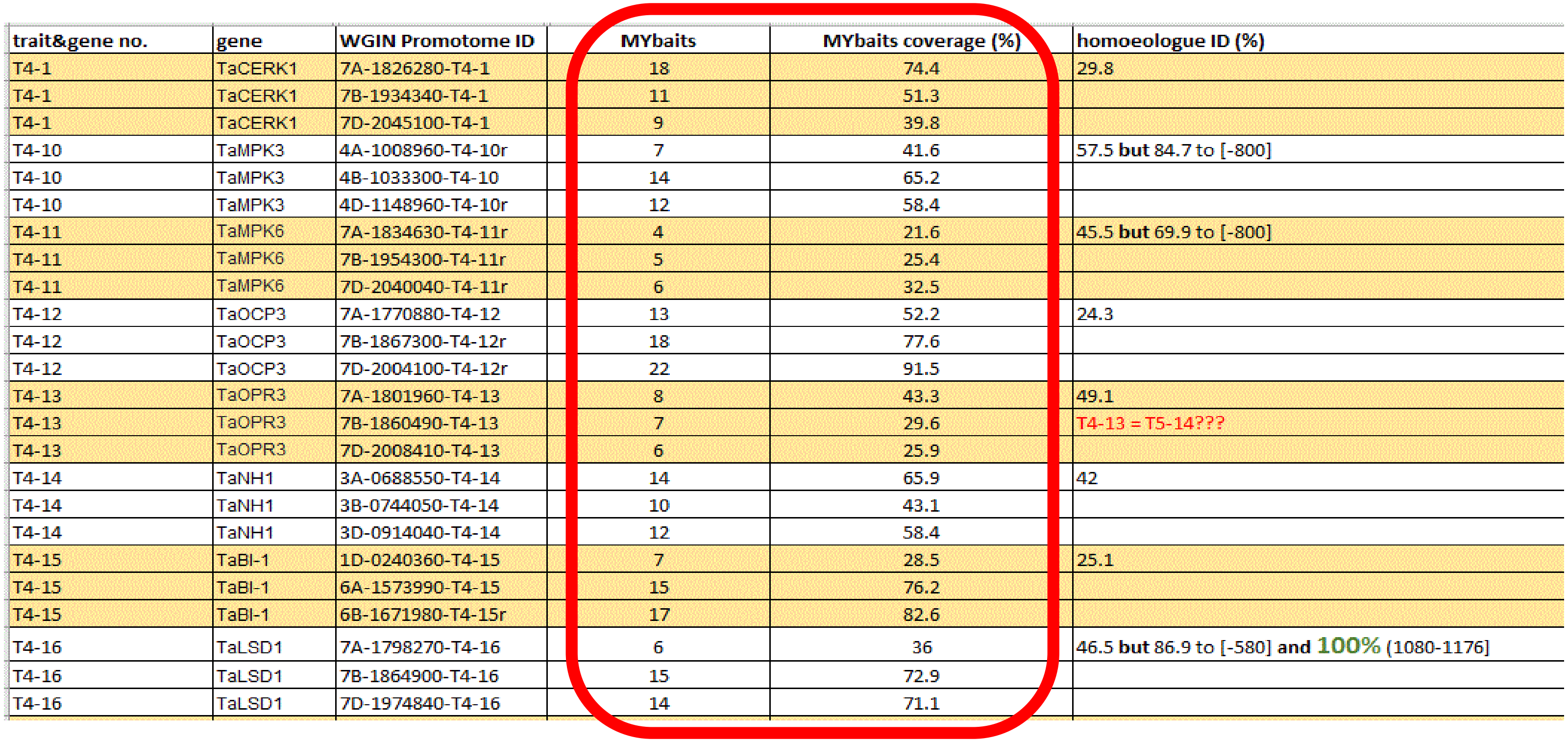
Average 10% = 1 genes per 10 Mbp

Average 1.84% = 18 – 19 genes per Mbp

MYbaits filtration to find baits (primers) **specific for each homoeologue**

MYbaits Coverage

How many baits are specific for each individual promoter sequence?



trait&gene no.	gene	WGIN Promotome ID	MYbaits	MYbaits coverage (%)	homoeologue ID (%)
T4-1	TaCERK1	7A-1826280-T4-1	18	74.4	29.8
T4-1	TaCERK1	7B-1934340-T4-1	11	51.3	
T4-1	TaCERK1	7D-2045100-T4-1	9	39.8	
T4-10	TaMPK3	4A-1008960-T4-10r	7	41.6	57.5 **but** 84.7 to [-800]
T4-10	TaMPK3	4B-1033300-T4-10	14	65.2	
T4-10	TaMPK3	4D-1148960-T4-10r	12	58.4	
T4-11	TaMPK6	7A-1834630-T4-11r	4	21.6	45.5 **but** 69.9 to [-800]
T4-11	TaMPK6	7B-1954300-T4-11r	5	25.4	
T4-11	TaMPK6	7D-2040040-T4-11r	6	32.5	
T4-12	TaOCP3	7A-1770880-T4-12	13	52.2	24.3
T4-12	TaOCP3	7B-1867300-T4-12r	18	77.6	
T4-12	TaOCP3	7D-2004100-T4-12r	22	91.5	
T4-13	TaOPR3	7A-1801960-T4-13	8	43.3	49.1
T4-13	TaOPR3	7B-1860490-T4-13	7	29.6	T4-13 = T5-14???
T4-13	TaOPR3	7D-2008410-T4-13	6	25.9	
T4-14	TaNH1	3A-0688550-T4-14	14	65.9	42
T4-14	TaNH1	3B-0744050-T4-14	10	43.1	
T4-14	TaNH1	3D-0914040-T4-14	12	58.4	
T4-15	TaBI-1	1D-0240360-T4-15	7	28.5	25.1
T4-15	TaBI-1	6A-1573990-T4-15	15	76.2	
T4-15	TaBI-1	6B-1671980-T4-15r	17	82.6	
T4-16	TaLSD1	7A-1798270-T4-16	6	36	46.5 **but** 86.9 to [-580] **and** 100% (1080-1176]
T4-16	TaLSD1	7B-1864900-T4-16	15	72.9	
T4-16	TaLSD1	7D-1974840-T4-16	14	71.1	

Mybaits Filtration

(performed by Dr. Jacob Enk @ MYcroarray)

1

- WGIN_Promotome_FASTA – 1,402 unique entry IDs, **1373* unique sequences**:
- **RepeatMasked** this using the cross-match algorithm and employing the Triticum repeat library available at RepeatMasker.org

2

- used default bait length and tiling configuration (**120nt baits**, tiled each 60nt, or "2X" bait coverage)
- Generates **36,352 baits** sequences, >35K unique.

3

- Filtration of baits for specificitythe by eliminating baits that
  (1) **map to multiple locations** in TGAC genome build with strong expected hybridization and/or
  (2) were **25% or more RepeatMasked**.

4

- A final **18,112 baits** survived this filtration (**17,745 unique***)

* differences: same gene ID provided for multiple traits -> seqs removed from all but one trait

Duplicate Gene IDs

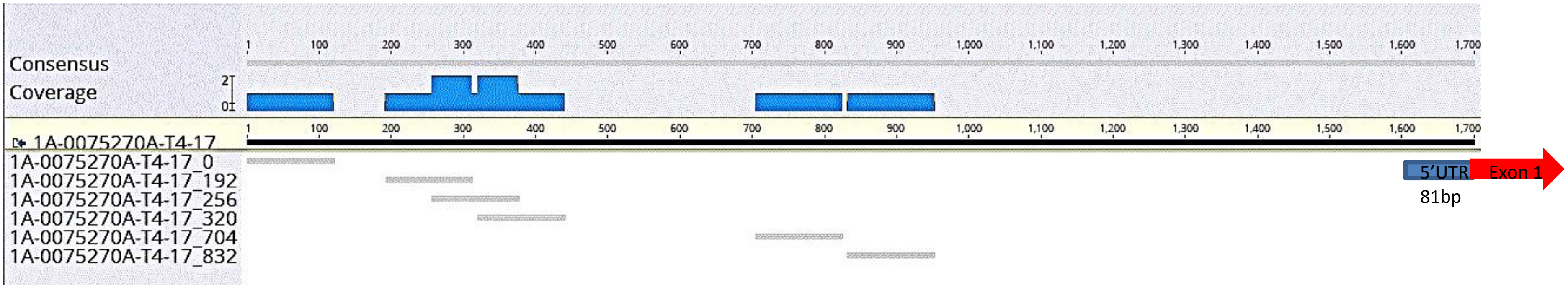
1) T1-3 = T6-41
2) T1-5 = T8-17
3) T2-2 = T3-13
4) T2-3 = T3-12
5) T2-5 = T3-17
6) T2-13 = T3-25
7) T2-27 = T3-27
8) T2-29 = T3-26
9) T2-35 = T3-23
10) T2-37 = T3-16
11) T2-39 = T3-24
12) T2-43 = T3-30
13) T2-45 = T3-31
14) T2-47 = T3-32
15) T2-49 = T3-33
16) T2-50 = T3-34
17) T2-51 = T3-35
18) T2-52 = T3-36 = T9-67
19) T2-53 = T3-28
20) T2-54 = T3-29
21) T2-56 = T3-39
22) T2-57 = T3-40
23) T2-58 = T3-42
24) T2-59 = T3-43
25) T2-60 = T3-44
26) T2-61 = T3-22
27) T6-1 = T9-63
28) T6-3 = T9-64
29) T6-5 = T9-58
30) T6-29 = T8-15
31) T6-30 = T8-16
32) T7-33 = T9-23
33) T7-36 = Ppd-A1 (control)
34) T7-53 = T8-27

MYbaits filtration for 1A-0075270A-T4-17

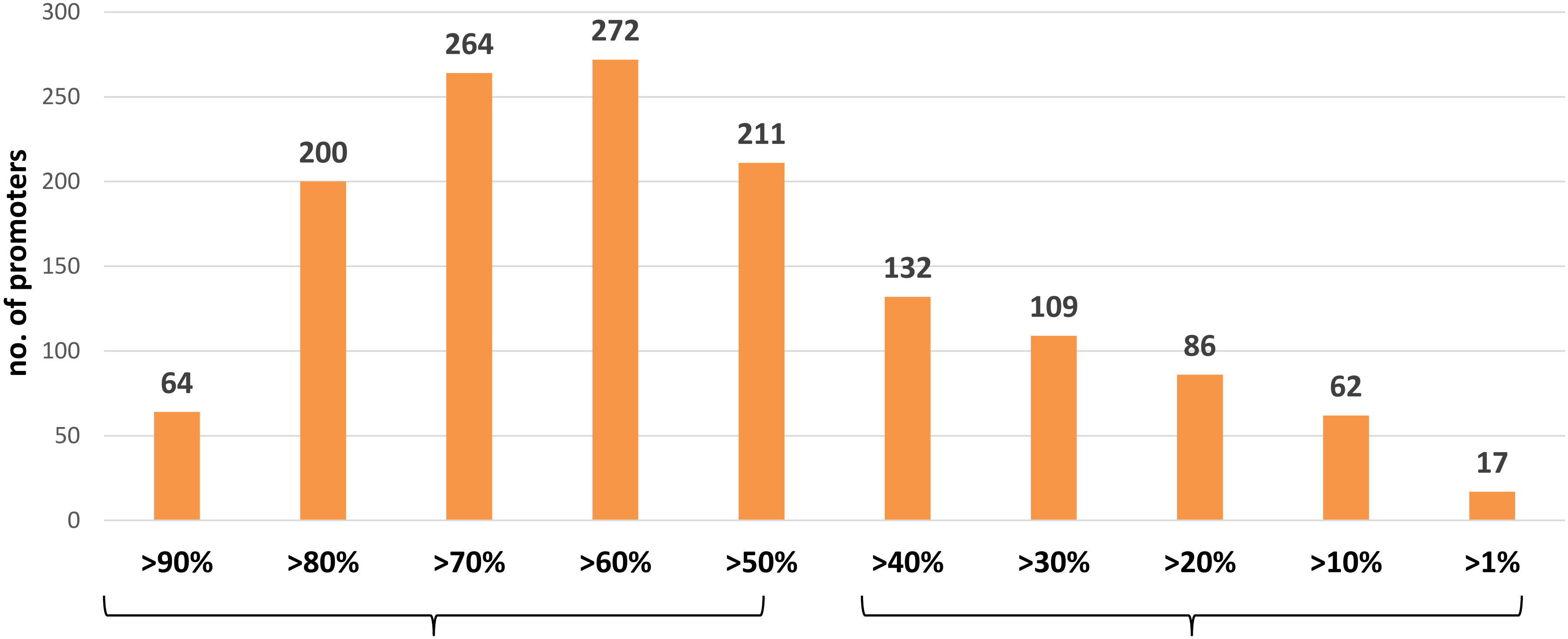
only 6 Baits pass stringent criteria

Bait	%GC	%RM	BLAST-Hits	40-60C	60-62.5C	62.5-65C	65-67.5C	67.5-70C	>70C	Stringent	Moderate	Relaxed	Sequen
1A-0075270A-T4-17_0	35	0	32	17	0	6	0	0	0	pass	pass	pass	TGCTATGATG
1A-0075270A-T4-17_64	34.2	0	25	13	1	7	1	0	0	fail	pass	pass	AAATATTTTCA
1A-0075270A-T4-17_128	35.8	0	40	31	1	6	0	0	0	fail	fail	fail	CCTCTATTCTC
1A-0075270A-T4-17_192	34.2	0	39	25	1	8	0	0	0	pass	pass	pass	TACTTGTATTT
1A-0075270A-T4-17_256	29.2	0	21	9	5	4	0	0	0	pass	pass	pass	AATCTAGGTAT
1A-0075270A-T4-17_320	29.2	0	22	8	9	3	0	0	0	pass	pass	pass	ATGAGGATAA
1A-0075270A-T4-17_384	40.8	0	33	15	2	1	6	3	0	fail	fail	fail	CATTAATGCTA
1A-0075270A-T4-17_448	39.2	0	15	3	1	4	6	0	0	fail	fail	fail	AAAAGTGCTT
1A-0075270A-T4-17_512	39.2	0	23	7	0	2	6	4	0	fail	fail	fail	TTTCAGATTCT
1A-0075270A-T4-17_576	45.8	0	17	2	0	0	1	10	1	fail	fail	fail	CAAAACCTTTG
1A-0075270A-T4-17_640	41.7	0	14	0	2	3	8	0	0	fail	fail	fail	TCTACTCACAT
1A-0075270A-T4-17_704	35	0	15	1	8	5	0	0	0	pass	pass	pass	TTACTTGCAAT
1A-0075270A-T4-17_768	35	0	22	3	2	6	6	0	0	fail	fail	fail	TAACTTATATC
1A-0075270A-T4-17_832	34.2	0	43	18	4	9	0	0	0	pass	pass	pass	GATACCAGTTT
1A-0075270A-T4-17_896	40.8	0	16	1	0	1	6	7	0	fail	fail	fail	AAAAATGGAG
1A-0075270A-T4-17_960	42.5	0	18	3	0	2	8	4	0	fail	fail	fail	GGAGAAGGC
1A-0075270A-T4-17_1024	37.5	0	21	6	1	5	8	0	0	fail	fail	fail	ATACAGTTCC
1A-0075270A-T4-17_1088	36.7	0	29	4	1	6	10	0	0	fail	fail	fail	AAGAAGAAGC
1A-0075270A-T4-17_1152	40.8	0	23	5	0	5	8	4	0	fail	fail	fail	AGCTGCTGCA
1A-0075270A-T4-17_1216	41.7	0	25	8	4	9	3	0	0	fail	fail	pass	GAGGTGGTTA
1A-0075270A-T4-17_1280	39.2	0	25	6	3	14	1	0	0	fail	fail	fail	AGGTGGCTAA
1A-0075270A-T4-17_1344	36.7	0	24	4	4	9	6	0	0	fail	fail	fail	TGAATTTCTTA
1A-0075270A-T4-17_1408	35.8	0	27	5	7	13	0	0	0	fail	fail	fail	AATGTCATTG
1A-0075270A-T4-17_1472	36.7	0	29	8	6	13	1	0	0	fail	fail	fail	TCTTATGCAG
1A-0075270A-T4-17_1536	39.2	0	21	1	7	12	0	0	0	fail	fail	fail	AAAGTTGATCT
1A-0075270A-T4-17_1580	47.5	0	3	0	0	0	1	1	0	fail	fail	fail	CAAGGGCACT

T4-17 (TaEDR1) A homoeologue – 38.5% Mybaits coverage



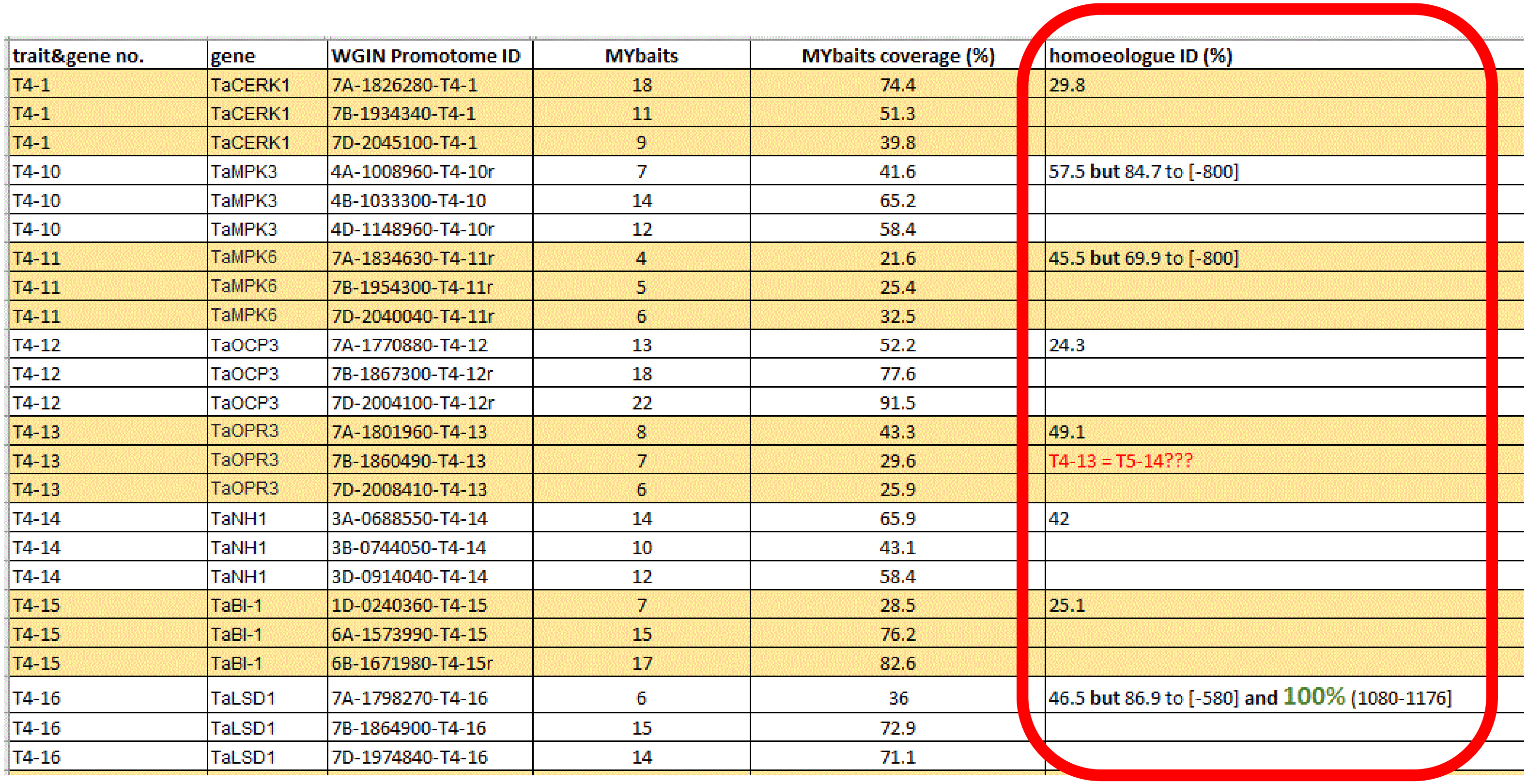
WGIN Promotome MYbaits coverage



71% of promoters > 50% coverage

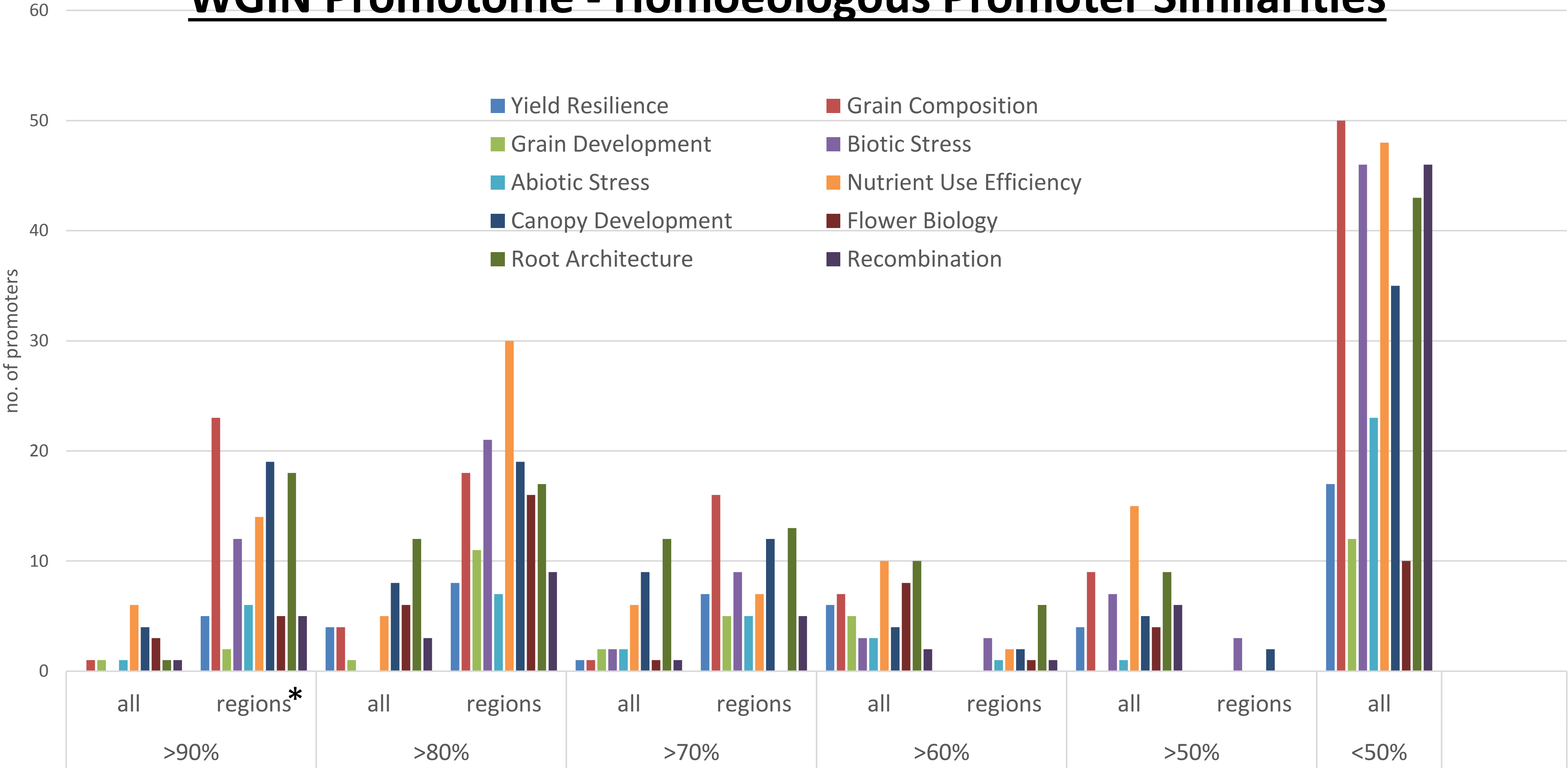
29% of promoters < 50% coverage

Comparison of Homoeologous Promoter Sequences



trait&gene no.	gene	WGIN Promotome ID	MYbaits	MYbaits coverage (%)	homoeologue ID (%)
T4-1	TaCERK1	7A-1826280-T4-1	18	74.4	29.8
T4-1	TaCERK1	7B-1934340-T4-1	11	51.3	
T4-1	TaCERK1	7D-2045100-T4-1	9	39.8	
T4-10	TaMPK3	4A-1008960-T4-10r	7	41.6	57.5 **but** 84.7 to [-800]
T4-10	TaMPK3	4B-1033300-T4-10	14	65.2	
T4-10	TaMPK3	4D-1148960-T4-10r	12	58.4	
T4-11	TaMPK6	7A-1834630-T4-11r	4	21.6	45.5 **but** 69.9 to [-800]
T4-11	TaMPK6	7B-1954300-T4-11r	5	25.4	
T4-11	TaMPK6	7D-2040040-T4-11r	6	32.5	
T4-12	TaOCP3	7A-1770880-T4-12	13	52.2	24.3
T4-12	TaOCP3	7B-1867300-T4-12r	18	77.6	
T4-12	TaOCP3	7D-2004100-T4-12r	22	91.5	
T4-13	TaOPR3	7A-1801960-T4-13	8	43.3	49.1
T4-13	TaOPR3	7B-1860490-T4-13	7	29.6	T4-13 = T5-14???
T4-13	TaOPR3	7D-2008410-T4-13	6	25.9	
T4-14	TaNH1	3A-0688550-T4-14	14	65.9	42
T4-14	TaNH1	3B-0744050-T4-14	10	43.1	
T4-14	TaNH1	3D-0914040-T4-14	12	58.4	
T4-15	TaBI-1	1D-0240360-T4-15	7	28.5	25.1
T4-15	TaBI-1	6A-1573990-T4-15	15	76.2	
T4-15	TaBI-1	6B-1671980-T4-15r	17	82.6	
T4-16	TaLSD1	7A-1798270-T4-16	6	36	46.5 **but** 86.9 to [-580] **and** 100% (1080-1176]
T4-16	TaLSD1	7B-1864900-T4-16	15	72.9	
T4-16	TaLSD1	7D-1974840-T4-16	14	71.1	

WGIN Promotome - Homoeologous Promoter Similarities



* regions ≥ 100bp

T9-11 (Bonzai 3 – copine family)

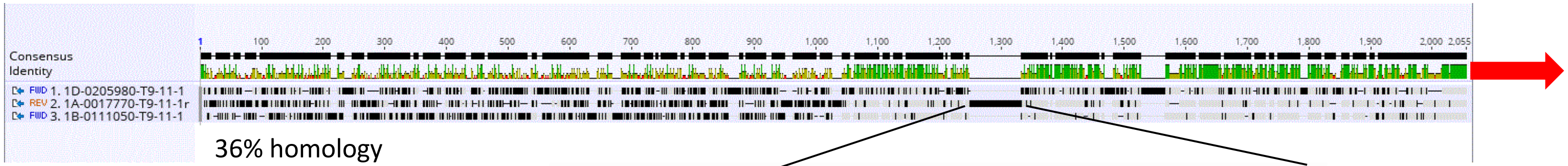
37,052 (on scaffold) = 418,057,131 (on Chr 1A, reverse strand)

39,487 (on scaffold) = 418,054,696 (on Chr 1A, reverse strand)

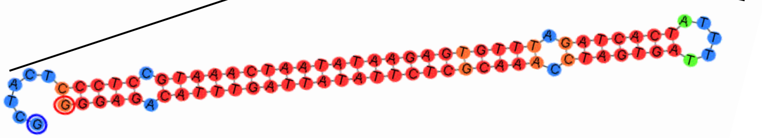
T9-11 ctd.

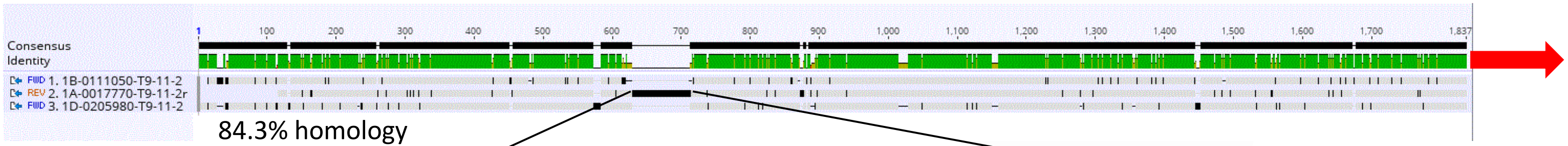
Both alternate promoters have **strikingly similar insertions in A homoeologue** with

- Similar position from ATG
- Very similar lengths (83 & 85bp) & 61% homology
- Very similar 2° structure (Palindromic)

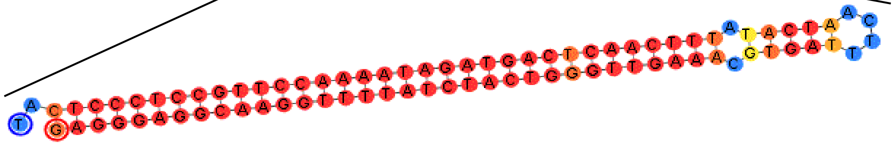


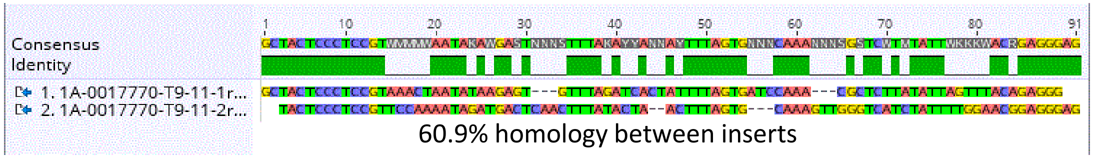
36% homology





84.3% homology



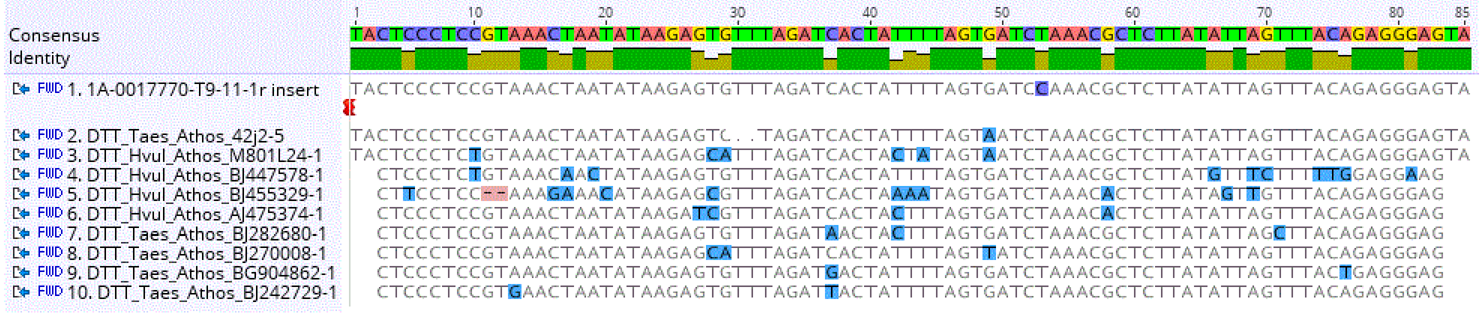


```
Consensus  GCTACTCCCTCCGTWMMMWAATAKAWGASTNNNSTTTAKAYYANNAYTTTAGTGNNNCAAANNNSGSTCWTMTATTWKKKWACRGAGGGAG
Identity
1. 1A-0017770-T9-11-1r...  GCTACTCCCTCCGTAAACTAATATAAGAGT---GTTTAGATCACTATTTTAGTGATCCAAA---CGCTCTTATATTAGTTTACAGAGGG
2. 1A-0017770-T9-11-2r...    TACTCCCTCCGTTCCAAAATAGATGACTCAACTTTATACTA--ACTTTAGTG---CAAAGTTGGGTCATCTATTTTGGAACGGAGGGAG
```

60.9% homology between inserts

1A-#-T9-11-1r insert has 99% identity to Taes Athos

(DNA-transposon, TIR, Mariner; Stowaway MITE, complete element)



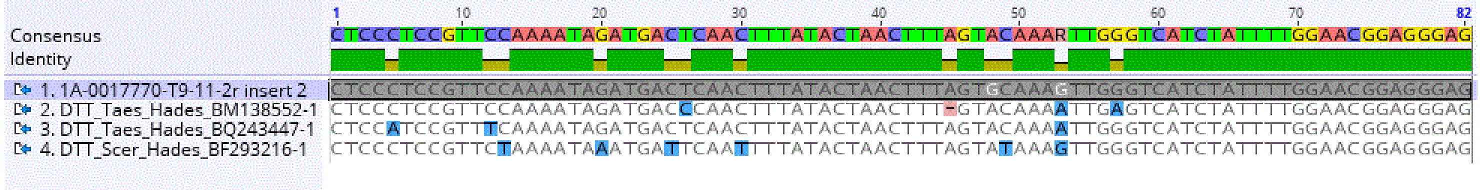
```
Consensus                             TACTCCCTCCGTAAACTAATATAAGAGTGTTTAGATCACTATTTTAGTGATCTAAACGCTCTTATATTAGTTTACAGAGGGAGTA
Identity
FWD 1. 1A-0017770-T9-11-1r insert     TACTCCCTCCGTAAACTAATATAAGAGTGTTTAGATCACTATTTTAGTGATCCAAACGCTCTTATATTAGTTTACAGAGGGAGTA

FWD 2. DTT_Taes_Athos_42j2-5           TACTCCCTCCGTAAACTAATATAAGAGTC. . .TAGATCACTATTTTAGTAATCTAAACGCTCTTATATTAGTTTACAGAGGGAGTA
FWD 3. DTT_Hvul_Athos_M801L24-1        TACTCCCTCTGTAAACTAATATAAGAGCATTTAGATCACTACTATAGTAATCTAAACGCTCTTATATTAGTTTACAGAGGGAGTA
FWD 4. DTT_Hvul_Athos_BJ447578-1         CTCCCTCTGTAAACAACTATAAGAGTGTTTAGATCACTATTTTAGTGATCTAAACGCTCTTATGTTTCTTTTTGGAGGAAG
FWD 5. DTT_Hvul_Athos_BJ455329-1         CTTCCTCC--AAAGAAACATAAGAGCGTTTAGATCACTAAAATAGTGATCTAAACACTCTTATAGTTGTTTACAGAGGGAG
FWD 6. DTT_Hvul_Athos_AJ475374-1         CTCCCTCCGTAAACTAATATAAGATCGTTTAGATCACTACTTTAGTGATCTAAACACTCTTATATTAGTTTACAGAGGGAG
FWD 7. DTT_Taes_Athos_BJ282680-1         CTCCCTCCGTAAACTAATATAAGAGTGTTTAGATAACTACTTTAGTGATCTAAACGCTCTTATATTAGCTTACAGAGGGAG
FWD 8. DTT_Taes_Athos_BJ270008-1         CTCCCTCCGTAAACTAATATAAGAGCATTTAGATCACTATTTTAGTTATCTAAACGCTCTTATATTAGTTTACAGAGGGAG
FWD 9. DTT_Taes_Athos_BG904862-1         CTCCCTCCGTAAACTAATATAAGAGTGTTTAGATGACTATTTTAGTGATCTAAACGCTCTTATATTAGTTTACTGAGGGAG
FWD 10. DTT_Taes_Athos_BJ242729-1        CTCCCTCCGTGAACTAATATAAGAGTGTTTAGATTACTATTTTAGTGATCTAAACGCTCTTATATTAGTTTACAGAGGGAG
```

***TREP*, the TRansposable Elements Platform (http://botserv2.uzh.ch/kelldata/trep-db/blast/blastTREP.html)**

There are **6,330 occurances** of this MITE in the Wheat genome (IWGSC), but **only 1 exact copy** with 85/85 identity (= 1A-0017770-T9-11-1r location)

1A-#-T9-11-2r insert has up to 98% identity to Taes Hades

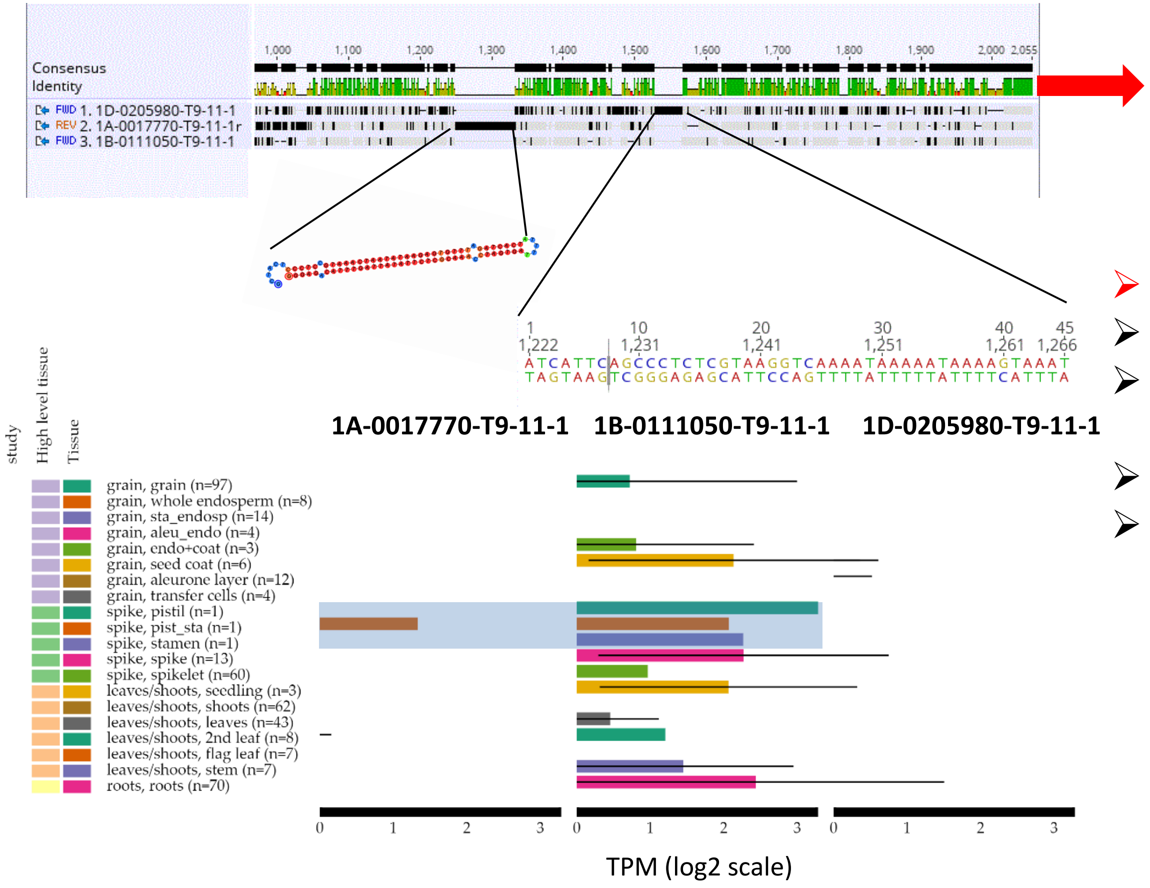
(DNA-transposon, TIR, Mariner; Stowaway MITE, complete element)



```
Consensus                           CTCCCTCCGTTCCAAAATAGATGACTCAACTTTATACTAACTTTAGTACAAARTTGGGTCATCTATTTTGGAACGGAGGGAG
Identity
1. 1A-0017770-T9-11-2r insert 2     CTCCCTCCGTTCCAAAATAGATGACTCAACTTTATACTAACTTTAGTGCAAAGTTGGGTCATCTATTTTGGAACGGAGGGAG
2. DTT_Taes_Hades_BM138552-1        CTCCCTCCGTTCCAAAATAGATGACCCAACTTTATACTAACTTT-GTACAAAATTGAGTCATCTATTTTGGAACGGAGGGAG
3. DTT_Taes_Hades_BQ243447-1        CTCCATCCGTTTCAAAATAGATGACTCAACTTTATACTAACTTTAGTACAAAATTGGGTCATCTATTTTGGAACGGAGGGAG
4. DTT_Scer_Hades_BF293216-1        CTCCCTCCGTTCTAAAATAAATGATTCAATTTTATACTAACTTTAGTATAAAGTTGGGTCATCTATTTTGGAACGGAGGGAG
```

There are **4,969 occurances** of this MITE in the Wheat genome (IWGSC) with **3 exact copies** on Chr 1A (= 1A-0017770-T9-11-1r location), 2A & 2B

T9-11-1 Homoeologues Expression Patterns



- **Only 1B homoeologue active**
- **1A** contains **Taes Athos**
- **1D** contains 45bp insert (w/o 2◦ structure) – but is **unique to this location on Chr1D**
- expression in **non-root tissues**
- expression levels highly variable

Expression graph modified from **Wheat Expression Browser** (Cristobal Uauy (JIC) http://www.wheat-expression.com)

1A-0017770-T9-11-1r & -2r inserts

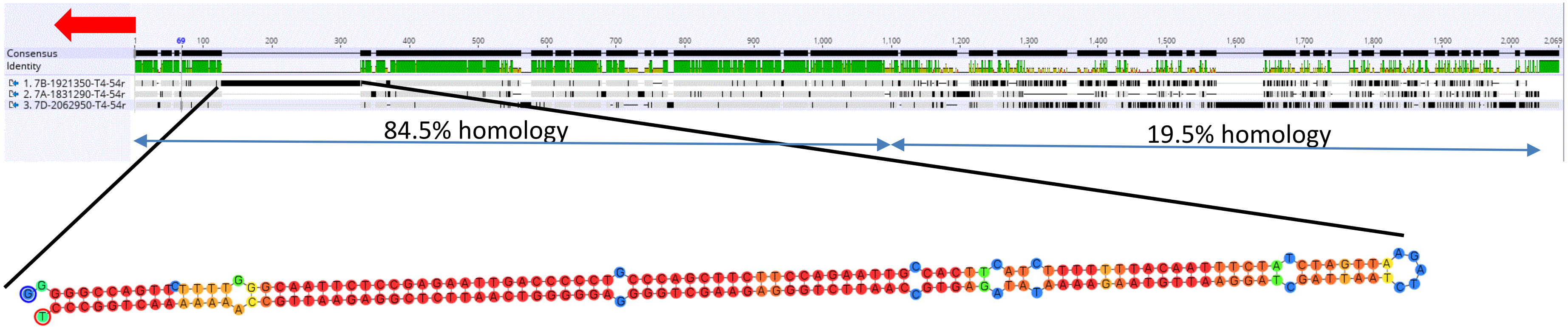
b) NCBI BLAST against ALL species (Plants, Fungi, Animalia etc):

ONLY hits in Pooideae → highly grass-specific MITEs

Genus	**Pooideae**	(largest subfamily of the grass family Poaceae)
Triticum aestivum	Triticinae	Familia: Poaceae
Aegilops tauschii subsp. tauschii		Subfamilia: Pooideae
Aegilops sharonensis		Tribus: Hordeeae
Aegilops longissima		Subtribus: **Triticinae**
Triticum monococcum subsp. monococcum cultiv		Genera: ***Aegilops*** – *Dasypyrum* – *Thinopyrum* – ***Triticum***
Triticum urartu		
Aegilops bicornis		
Triticum dicoccoides		
Triticum turgidum subsp. dicoccon		
Triticum timopheevii subsp. armeniacum		
Aegilops speltoides Pina		
Secale cereale	Hordeinae	Familia: Poaceae
Agropyron cristatum		Subfamilia: Pooideae
Agropyron mongolicum		Tribus: Hordeeae
Hordeum vulgare		Subtribus: **Hordeinae**
Kengyilia melanthera		Genera: ***Agropyron*** – *Anthosachne* – *Australopyrum* – *Connorochloa*
Kengyilia grandiglumis		– *Crithopsis* – *Elymus* – *Eremopyrum* – *Festucopsis*
Kengyilia mutica		– *Henrardia* – *Heteranthelium* – *Hordelymus* – ***Hordeum***
Kengyilia longiglumis		– *Hystrix* – ***Kengyilia*** – *Leymus* – *Pascopyrum*
		– *Peridictyon* – *Psathyrostachys* – *Pseudoroegneria* – ***Secale***
		– *Stenostachys* – *Taeniatherum*

T4-54r (AK360626 – RING-H2 finger protein)

B homoeologue insertion with stable secondary structure (palindrome)

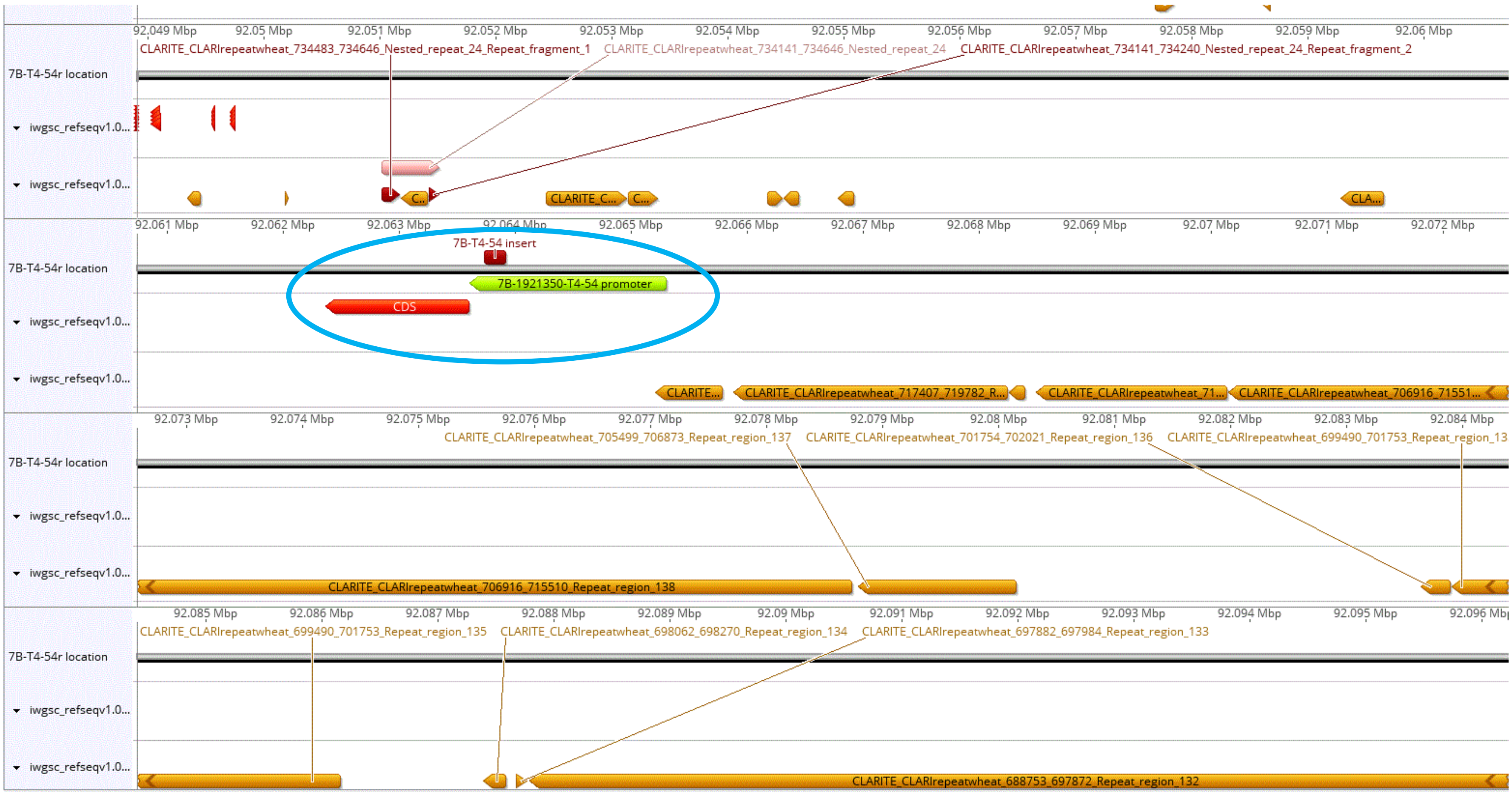


- **Very stable 2º structure**
- **Possible MITE ?**
- **1,020 hits** in Wheat Genome
- **NO match** in TREP database & IWGSC_refseqv1.0_TransposableElements_2017Mar13

➡ **New MITE?**

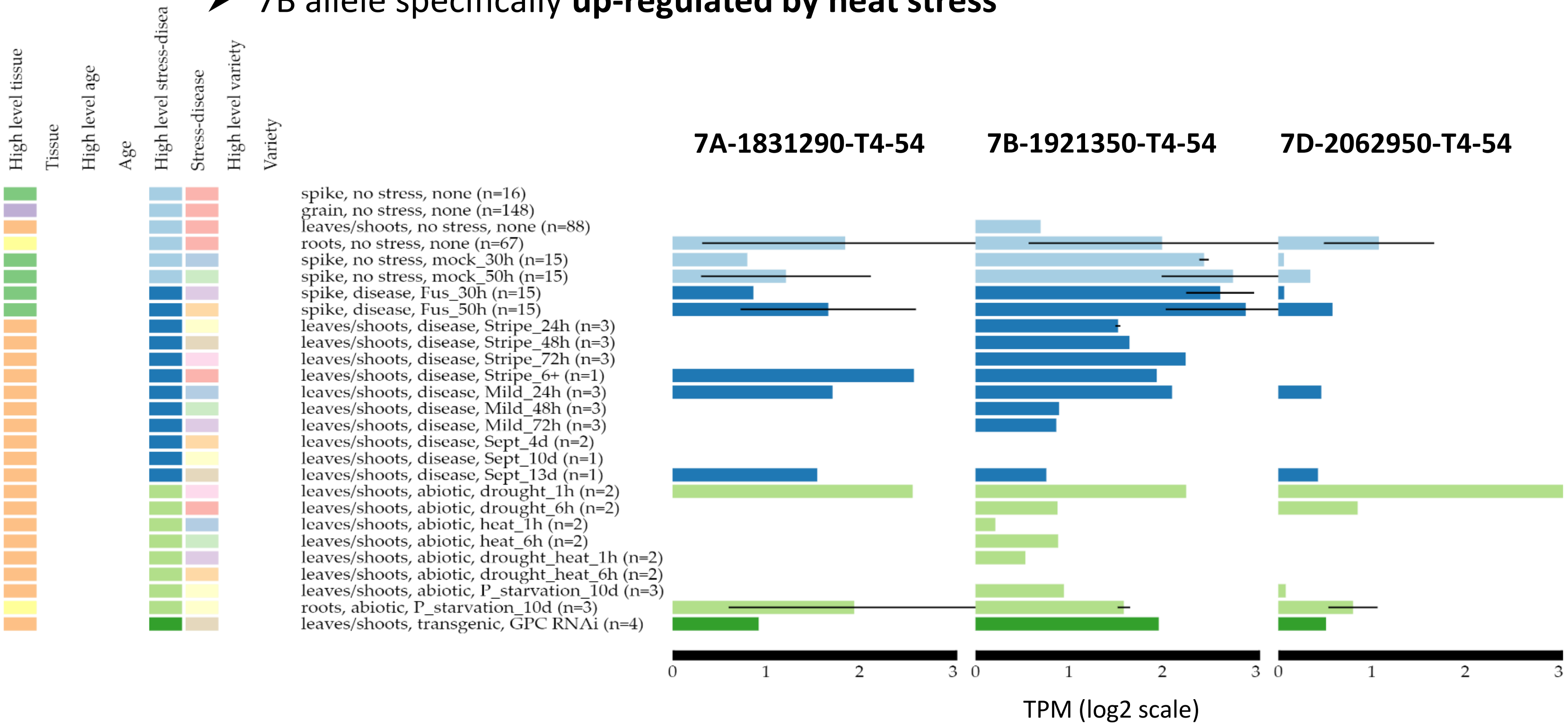
Chr 7B Map of 7B-1921350-T4-54r region

With Surrounding Repeat Regions/Fragments



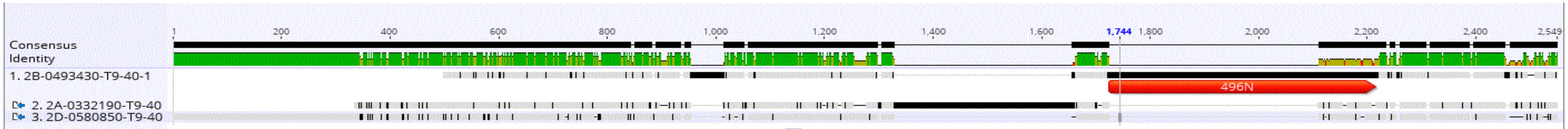
T4-54r homoeologue Expression Patterns

- Generally **Higher Expression levels on 7B** which contains the MITE
- 7B allele specifically **up-regulated by Yellow Rust & Mildew** (>48h)
- 7B allele specifically **up-regulated by heat stress**



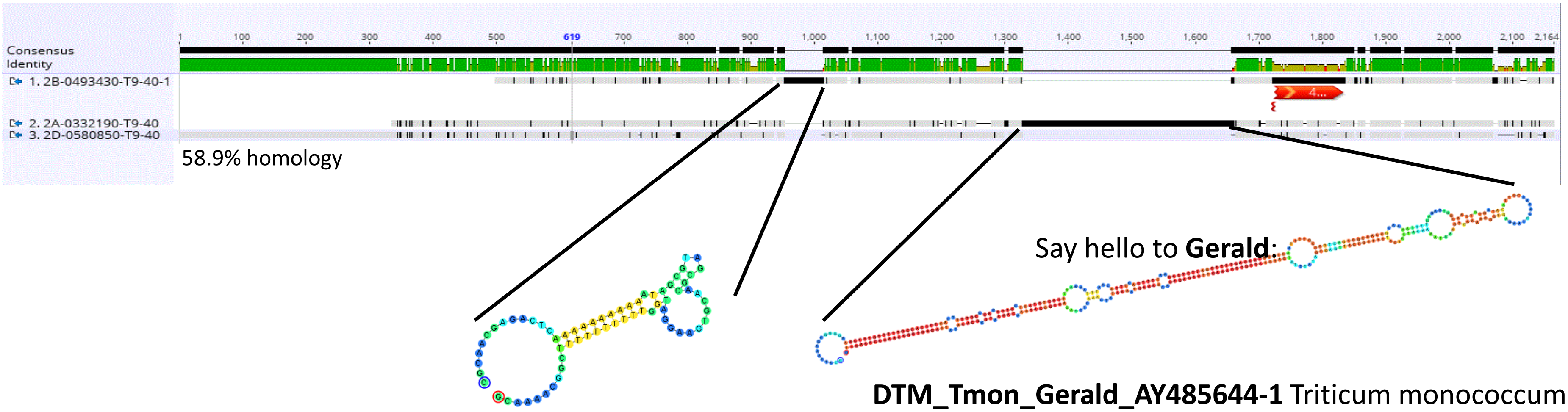
T9-40 (SGN3/GSO 1)

Precise number of Ns in IWGSC RefSeq v1.0 may be **too large?**



48.7% homology

Removal of **396Ns** from 2B-T9-40-1



58.9% homology

Say hello to **Gerald**:

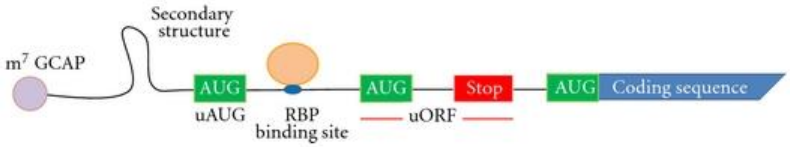
DTM_Tmon_Gerald_AY485644-1 Triticum monococcum &
DTM_Taes_Gerald_RND3B-1 Triticum aestivum
DNA-transposon, TIR, Mutator

5’ UTRs (UnTranslated Regions)

- The 5’ untranslated region (5’ UTR) (also known as a leader sequence or leader RNA) is the region of an mRNA that is directly upstream from the initiation codon. This region is **important for the regulation of translation of a transcript** by differing mechanisms in viruses, prokaryotes and eukaryotes.

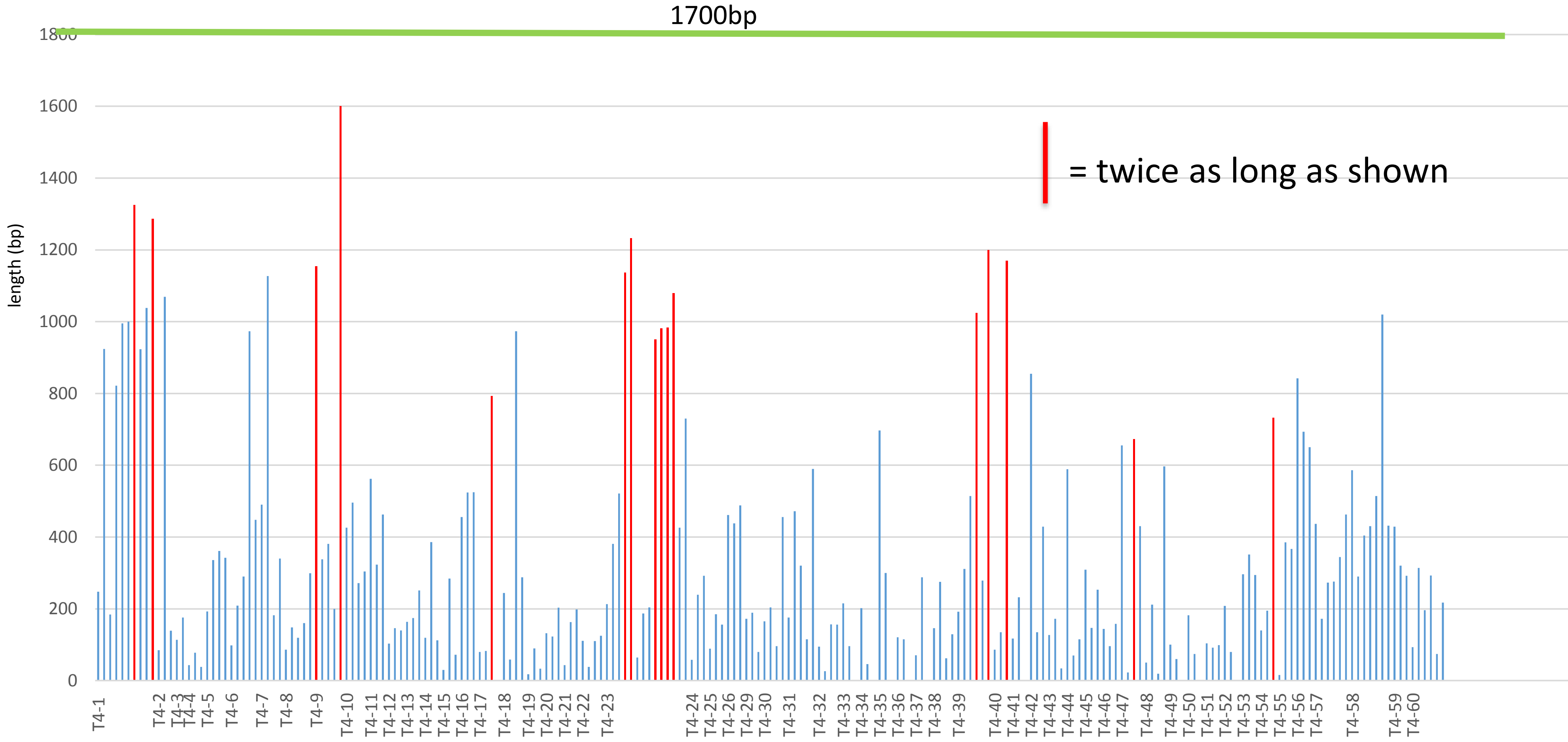
- Most promoter elements are not a part of the mRNA sequence. They are upstream (towards 5') of the transcription start site. However, a certain class of promoters called **downstream promoter elements** (DPE) can overlap with the genetic region. These elements have been reported to lie at 29-33bp upstream of the transcription start site and are widely employed in Drosophila. Mapping of DPEs has been done for mouse and human genes as well.

- There is also evidence for **small uORFs** (upstream Open Reading Frames) **inside the 5’UTRs that express peptides downregulating plant defense genes** (Guoyong Xu *et al* Nature 2017).



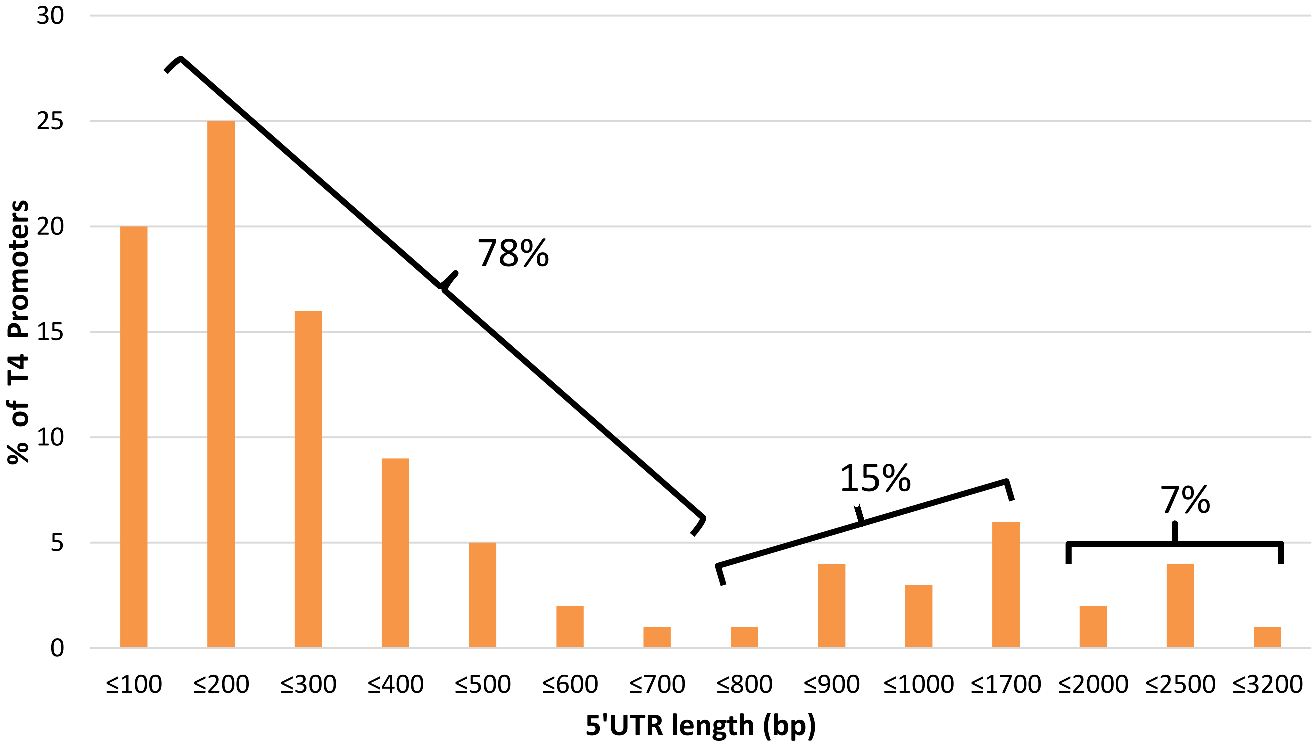
Generalised structure of mRNA

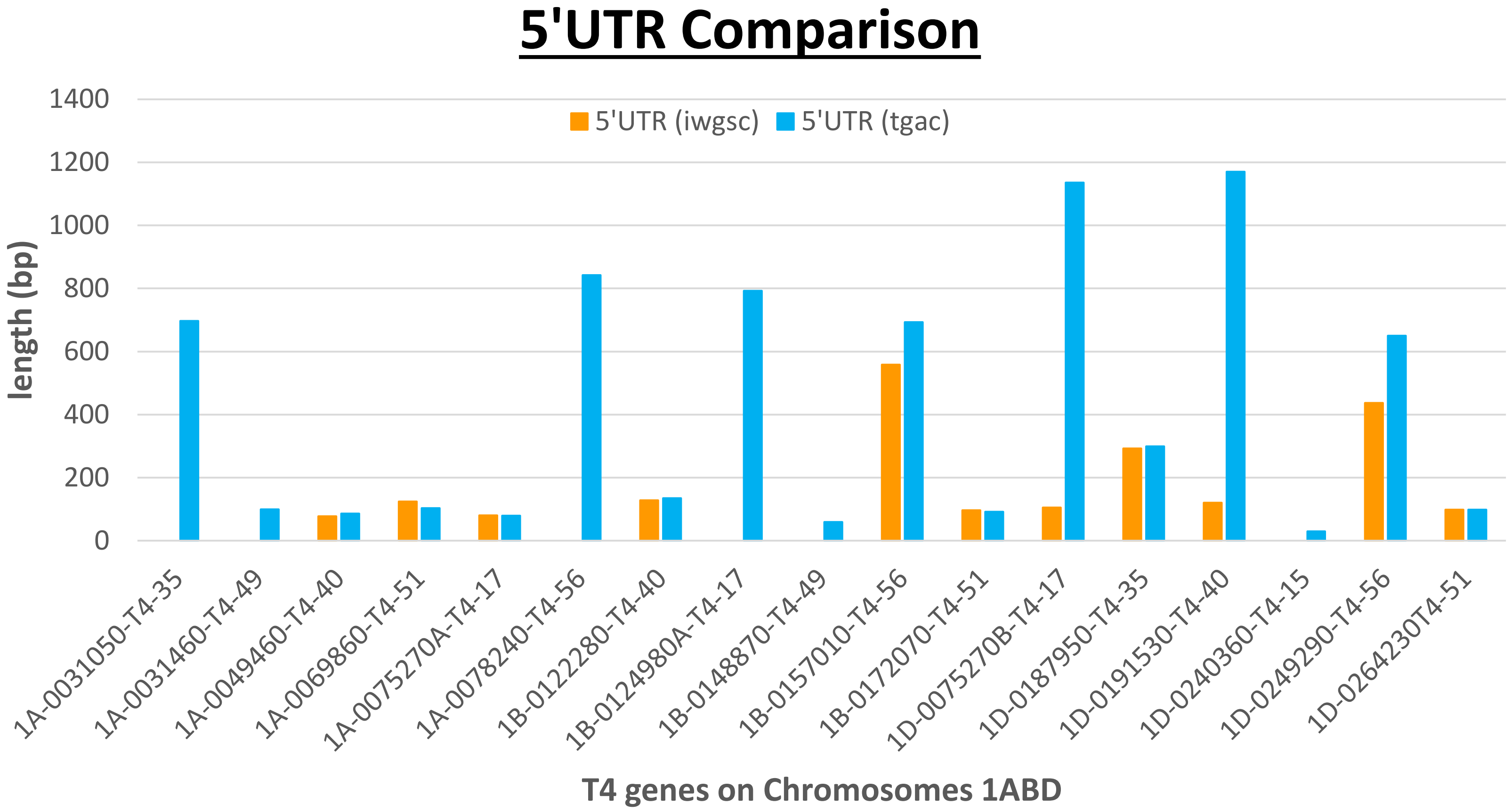
5'UTR length for Trait 4 (Biotic Stress) Genes



T4 Promoters Grouped by 5’UTR Length

- **78% of promoters will have at least 1000bp of promoter**
- **Only 7% would have no promoter sequence**




5'UTR Comparison
5'UTR (iwgsc)
5'UTR (tgac)
length (bp)
1400
1200
1000
800
600
400
200
0
1A-0031050-T4-35
1A-0031460-T4-49
1A-0049460-T4-40
1A-0069860-T4-51
1A-0075270A-T4-17
1A-0078240-T4-56
1B-0122280-T4-40
1B-0124980A-T4-17
1B-0148870-T4-49
1B-0157010-T4-56
1B-0172070-T4-51
1D-0075270B-T4-17
1D-0187950-T4-35
1D-0191530-T4-40
1D-0240360-T4-15
1D-0249290-T4-56
1D-0264230T4-51
T4 genes on Chromosomes 1ABD

Should We Change The Project Name – Again?

WGIN **Exome** Capture

WGIN **Promotome** Capture

WGIN **PromoUTRome?** Capture

Key Outcomes So Far – Prior to MYbaits Capture

50 genes were reassigned to different chromosomes (TGAC → IWGSC), in 32 cases completing the ABD Homoeologue sets

Very specific primers identified **for each homoeologue**

71% of promoters with >50% MYbaits coverage

→ **full 1700bp sequences expected** for ALL of these

New **MITE** identified (let's call it **Taes WGIN**) – by aligning the homoeologous promoter/5'UTR sequences

Good evidence for **differential homoeologous expression** in Chinese Spring – vindicating the WGIN approach of capturing individual homoeologues

Chromosomes with highest densities for ALL Traits are **7D, 7A & 1D** (243/1390)

Department
for Environment
Food & Rural Affairs

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Wheat
Genetic
Improvement
Network

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