

Tools, resources, genotyping and phenotyping (getting more precise in WGIN4!)

Clare Lister, Scott Sheldrick and Simon Griffiths

4/10/2018



Continuing WGIN3 Projects

1. Drought tolerance in Paragon x Garcia

2017-18 Trial

- No spring drought during grain number formation but drought during grain filling
- Data not yet analysed

2018-19 Trial

- Will be drilled mid-October



Continuing WGIN3 Projects

2. Paragon Library

- DNA and seed from single plants
- Genotyped on Axiom 35K Breeders Array
- Library lines and genotyping data to WGIN website
- Seed stocks ready for distribution



Continuing WGIN3 Projects

3. Chromosome Segment Substitution Library for A x C

- DNA and seed from single BCF2 plants (57 lines)
- Markers generated by Polymarker
- Genotyping with markers for substituted regions
Chr 1/2/3/4/5/6/7
- Genotyping data to WGIN website



WGIN4 Projects

1. Drought tolerance
2. Anchorage and lodging
3. Resistance to slug damage
4. CSSL
5. CSSL and ParLib data
6. Gene content around 2D, 3A and 6A
7. Promotome
8. Organise workshop on Yield Stability
9. QTL nominated to DFW Breeders Tool Kit
10. Curation and distribution of WGIN germplasm



WGIN4 Projects

1. Drought tolerance – selection of lines
2. Anchorage and lodging – selection of lines
3. Resistance to slug damage
4. CSSL (continuing from WGIN3)
5. CSSL and ParLib data to WGIN webpage when analysis complete(continuing from WGIN3)
6. Gene content around 2D, 3A and 6A
7. Promotome
8. Organise workshop on Yield Stability
9. QTL nominated to DFW Breeders Tool Kit – 2 drought QTL
10. Curation and distribution of WGIN germplasm – ongoing



WGIN4 Projects

1. Drought tolerance – selection of lines
 2. Anchorage and lodging – selection of lines
- Originally creating small collection about 50 lines but decided to get as many as possible from ‘loong list’ of lines sensitive or resistant to drought and / or lodging
 - Many thanks to the breeding companies and RothRes for providing seed
 - 1 m plots to bulk seed for yield trials in 2019/2020



WGIN4 Projects

Possible Drought and Lodging lines	RL crosses	Parent of other crosses	CIMMYT	RothRes	Possible Drought and Lodging lines	RL crosses	Parent of other crosses	CIMMYT	RothRes
Alchemy					LG Motown				
Atilla					LG Skyscraper				
Avalon					LG Sundance				
Baj					Lr19				
Barrel					Malacca				
Becard Kachu					Maris Widgeon				
Beluga					Mascani - OAT!				
Borlaug 100					MISR1				
Cadenza					Pamyati Azieva				
Charger					Panorama				
Chinese Spring					Paragon				
CIMCOG 47					Paragon EMS semi dwarves (x5)				
CIMCOG 49					Paragon Rht D1 x B1				
Claire					Paragon RhtB1				
Conqueror					Paragon RhtB1 x Rht8				
Cordiale					Paragon RhtD1				
Cordiale 3N (Rec 5-1)					Paragon RhtD1 x Rht8				
Costello					Pastor				
Cougar					Pfau				
Crusoe					Pomerelle				
Denman					Reflection				
DFW Breeder Toolkit H17 (x3)					Revelation				
DFW Breeder Toolkit H18 (x3)					RGT Illustrious				
Einstein					Rht8				
Fiorello					Riband				
Freiston					Robigus				
Gallant					Savello				
Garcia					Scout				
Glasgow					Siskin				
Grafton					Skyfall				
Graham					Soisson				
Hereward					Sokoll				
Horatio					Solstice				
Hylux					Spark				
Icon					Super 152				
Invicta					Synth Type				
Istabraq					Treasure				
JB Diego					Watkins 110				
KWS Croft					Watkins Indian dwarfs W126				
KWS Gator					Watkins Indian dwarfs W127				
KWS Kielder					Waxwing				
KWS Santiago					Weebill				
KWS Silverstone					Wyalkatchem				
KWS Siskin					Xi19				
KWS Sterling					Zyatt				
KWS Zyatt									



WGIN4 Projects

1. Drought tolerance – selection of lines
 2. Anchorage and lodging – selection of lines
- Preliminary anchorage tests on PxG...



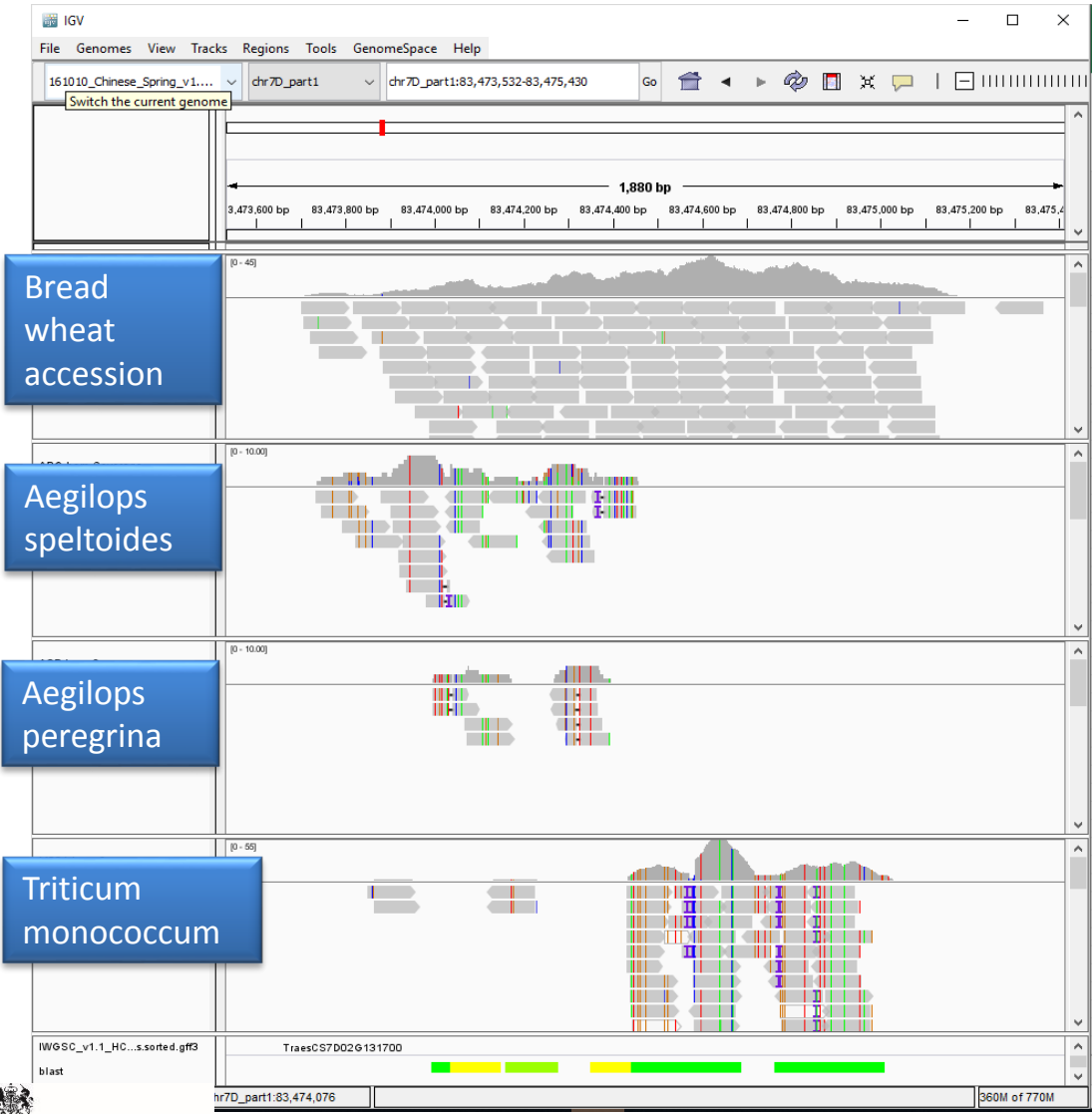
WGIN4 Projects

7. Promotome

- John Innes Undergraduate Summer student, [Scott Sheldrick](#)
- Project to assess the diversity within the promoters of flowering time genes (from Trait 7 and 8 lists) among the 96 wheat species and accessions
- To facilitate genotyping of other wheat accessions
- Sequences were aligned to the Chinese Spring reference
- Used **Integrative Genomics Viewer (IGV)** to analyse the data.



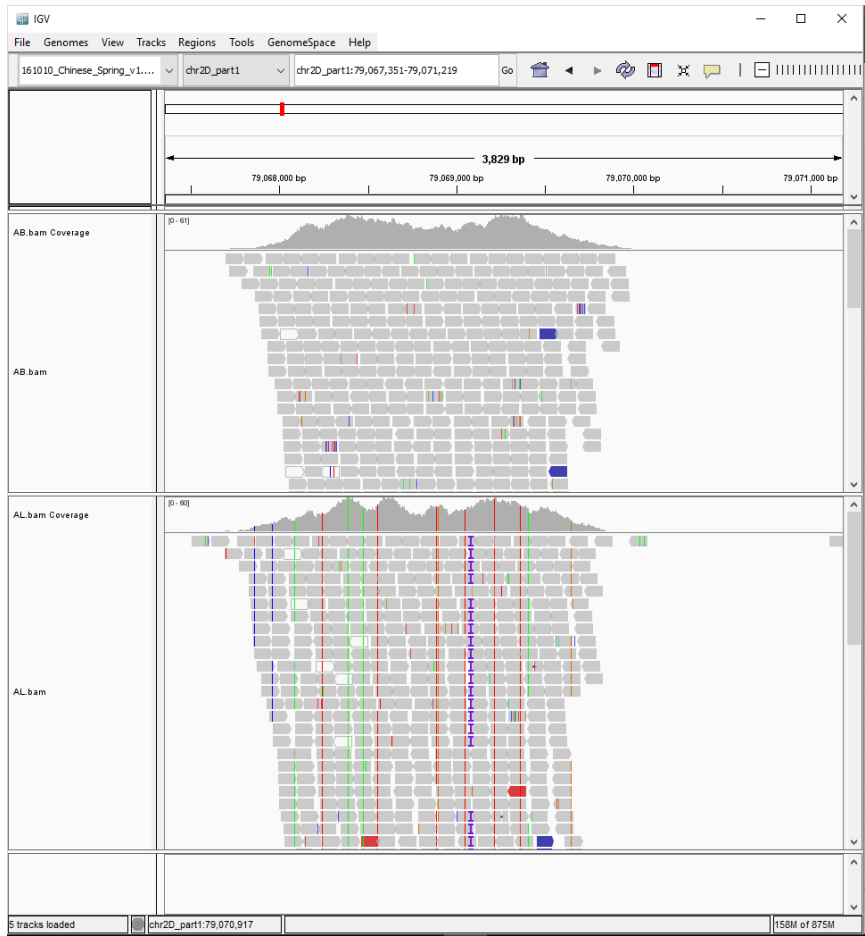
WGIN4 Projects: Promotome



- *CO8* promoter (7D)
- Not surprisingly more distantly related species had significantly altered promoter sequences.

WGIN4 Projects: Promotome

Sometimes get very clear data...



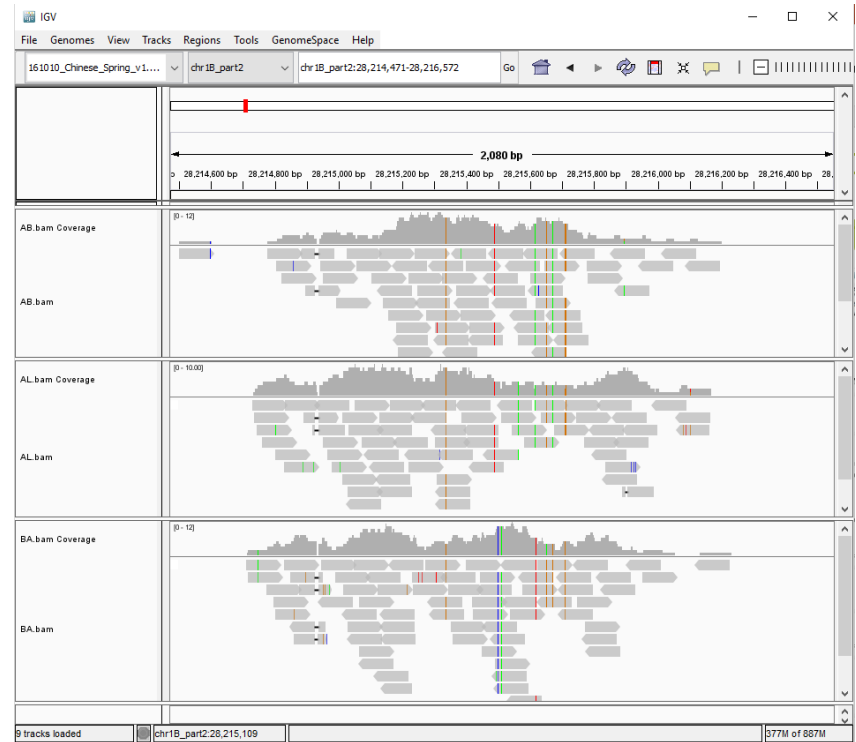
- *FT4* promoter (2D)
- i.e. obvious variation between accessions Abbot and Alcedo.
- 15 SNPs and one insertion.
- Every wheat accession either had the variant allele or not.

WGIN4 Projects : Promotome

... and sometimes not so clear



SOC1 promoter

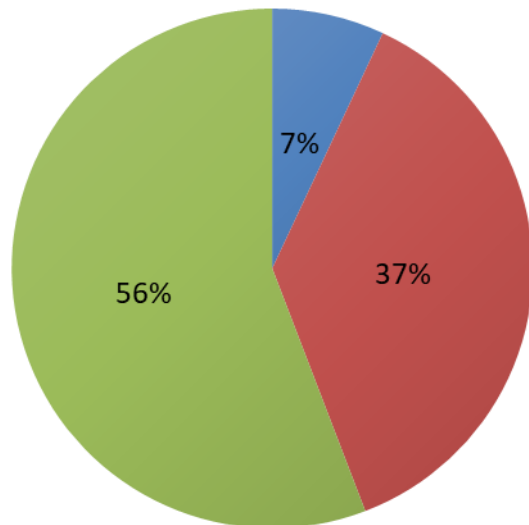


GID1 promoter

- Mis-binding of primers?
- Example from another project...
 - Added value or a complication?

WGIN4 Projects : Promotome

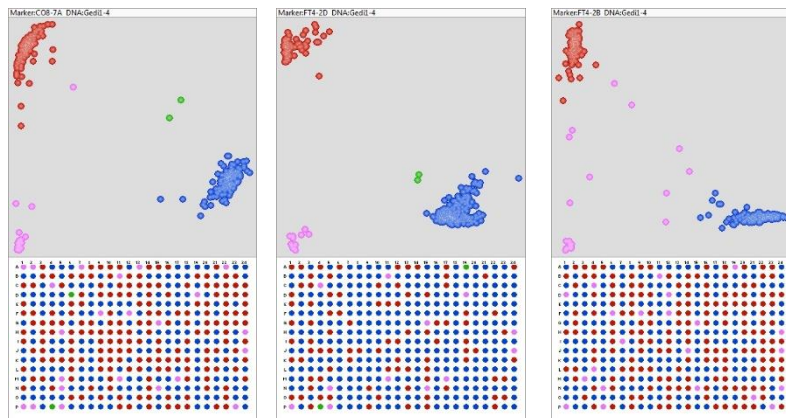
Classification	Number of promoter sets
Unusable	6
Clear variation	32
Complicated variation...	48
Total	86



Promoter	Number of alleles
AGL10 (2B)	3
AGLG1 (5A)	3
AGLG1 (5B)	3
CO2 (6A)	2
CO2 (6B)	4
CO7 (6B)	4
CO8 (7A)	2
ELF3 (1A)	6
FT1 (7A)	2
FT2 (3B)	2
FT3 (1B)	2
FT4 (2B)	2
FT4 (2D)	2
FT10 (5B)	3
FT10 (5D)	4
GA2ox3 (3A)	5
GA2ox3 (3D)	3
GA2ox4 (1A)	3
GA2ox4 (1B)	6
GA3ox1 (2A)	5
GA3ox1 (2B)	5
GID1 (1B)	4
GID2 (3A)	2
GID2 (3D)	7
PhyA (4A)	2
PhyC (5B)	2
Ppd1 (2A)	10
SOC1 (1A)	2
TOE1 (1A)	5
TOE1 (1B)	4
TOE1 (1D)	2
WCO1 (7A)	2

WGIN4 Projects : Promotome

- Used SNPs to manually design KASP markers for 19 of these genes
- Tested markers on Gediflux collection, 17/19 worked



	A	B	C	D	E	F	G	H
1	SubjectID	Co2-6A	Co2-6B	FT4-2B	FT4-2D	CO8-7A	TOE1-1A-4	
2	Peragis	DNA	AA	GG	AA	DNA	GA	
3	Admonter	AA	GG	TT	GG	GG	GG	
4	Bersee	AA	GG	GG	GG	AA	GG	
5	Agron	DNA	DNA	DNA	AA	GG	DNA	
6	Eroica	AA	AA	GG	AA	AA	GG	
7	Capo	AA	GG	GG	GG	AA	GG	
8	Thor	AA	AA	GG	AA	GG	GG	
9	Danubius	AA	GG	TT	GG	AA	GG	
10	Rimpaus Braur	AA	GG	GG	AA	AA	GG	
11	Drauhofener K	GG	AA	GG	GG	GG	GG	
12	Carstens 6	AA	GG	GG	AA	GG	GG	
13	Lindos	AA	AA	TT	GG	AA	GG	
14	Carstens 8	AA	GG	GG	GG	GG	GG	
15	Loosdorfer Aus	GG	AA	GG	GG	AA	GG	
16	Flamingo	AA	GG	GG	AA	GG	GG	
17	Schweigers Tax	DNA	DNA	DNA	DNA	DNA	DNA	
18	Arminda	DNA	GG	TT	AA	DNA	GA	
19	sirius	AA	GG	GG	GG	GG	GG	
20	Granta	AA	GG	TT	AA	AA	GG	
21	hanno	AA	GG	GG	GG	GG	GG	
22	Adam	GG	GG	GG	GG	GG	GG	
23	wizard	AA	GG	TT	GG	AA	GG	
24	Aquila	AA	GG	TT	GG	AA	GG	
25	baron	AA	GG	TT	GG	GG	GG	
26	Court't	AA	AA	GG	GG	GG	GG	
27	kronjuwel	AA	AA	GG	GG	AA	GG	
28	Mironowskaja	AA	AA	GG	AA	GG	GG	
29	guardian	AA	AA	TT	GG	GG	GG	
30	Nautica	AA	AA	TT	GG	AA	GG	
31	sabre	AA	GG	GG	GG	GG	GG	
32	Fakir	GG	AA	GG	AA	GG	GG	
33	renard	DNA	GG	GG	GG	DNA	GG	
34	Heine 7	DNA	GG	TT	GG	AA	GA	
35	Escorial	AA	GG	TT	AA	AA	GG	
36	Werla	AA	AA	GG	AA	GG	GG	
37	lena	AA	AA	GG	GG	GG	GG	

WGIN4 Projects : Promotome

- Association analysis performed to determine whether observed variation in *promoter genotypes* is associated with phenotypic *variation in height and flowering time*.
- Association with height and DTEM data assessed in the Gediflux collection, using a generalised linear model in the programme Tassel.
- From this the likelihood of a genotype-phenotype association was estimated.

WGIN4 Projects : Promotome

Several promoter genotypes showed significant association with flowering time and height phenotypes

Promoter	Associated Trait (Data year)	P-value
FT4 (2B)	Height (2011)	4.65e-6
	Height (2016)	7.74e-07
	DTEM (2011)	1.05e-3
	DTEM (2016)	0.01026
FT4 (2D)	DTEM (2016)	8.6e-3
TOE (1A)	DTEM (2016)	0.01029
CO2 (6A)	Height (2016)	0.03645
AGL10 (2B)	DTEM (2011)	0.02243
AGLG1 (5A)	Height (2016)	0.02049
CO7 (6B)	Height (2011)	0.03779
	DTEM (2016)	0.04408

WGIN4 Projects : Promotome

Lots to do after drilling!

- Analyse PxG 2018 data
- CSSL genotyping and data
- Paragon library information and data
- Continuing Promotome analysis
- Gene content around 2D, 3A and 6A, now sequence publicly available



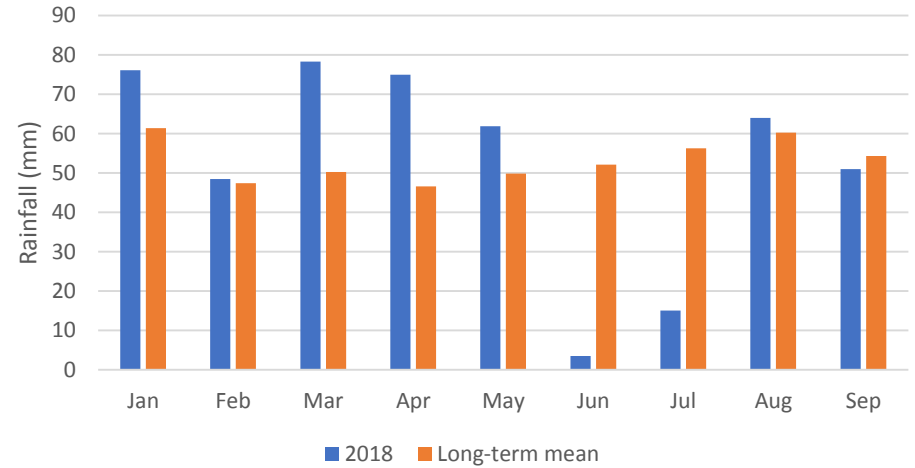
Wgin Diversity Trial Update



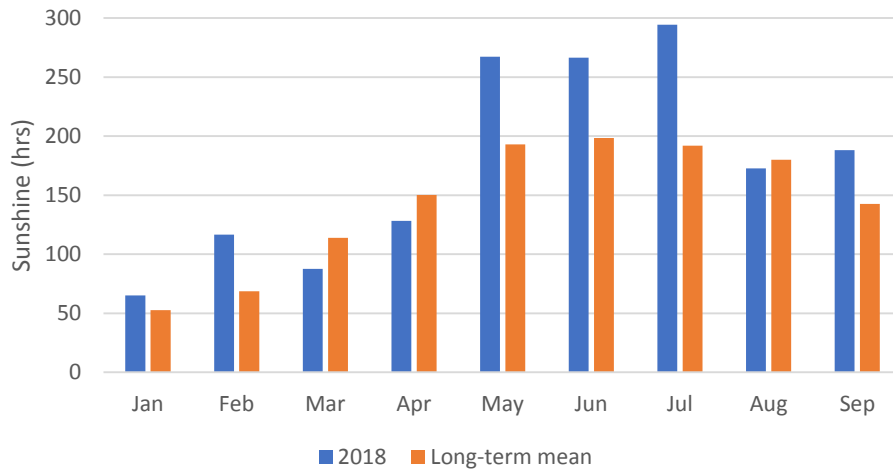
2018 Weather

- June – July very dry
- May – July very sunny
- Temp exceeded 30 degrees on 5 days, late July-early Aug

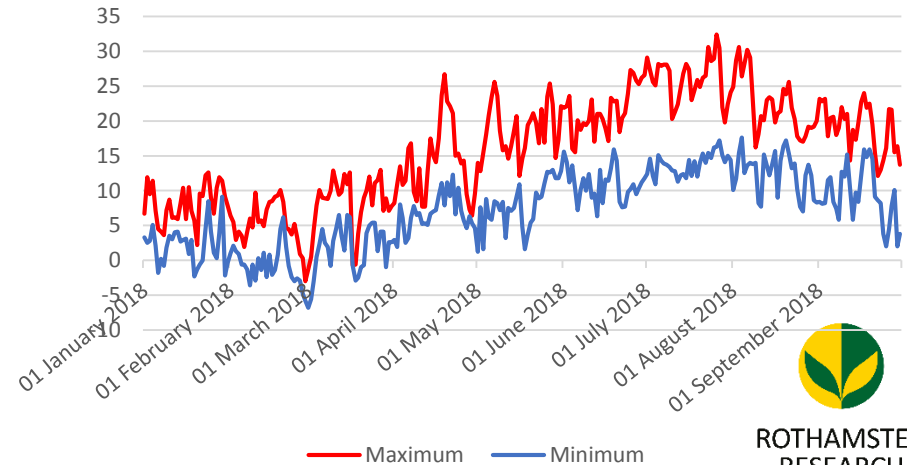
Rothamsted Monthly Rainfall



Rothamsted Monthly Sunshine



2018 Rothamsted daily temperature



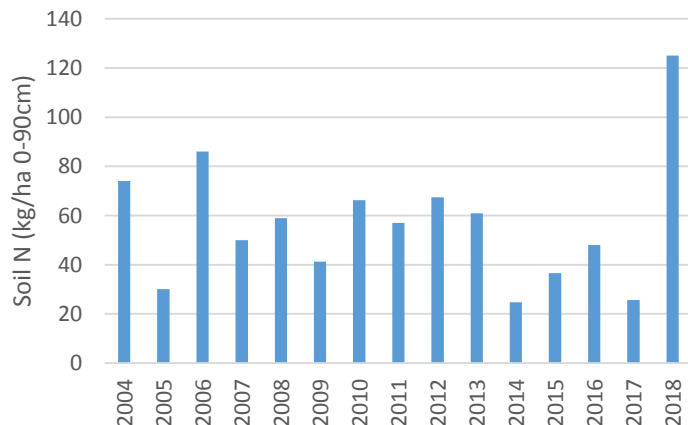
2018 Yields

- Sown 1/11/17
- N applied in two applications, mid-Apr and 04/05/18

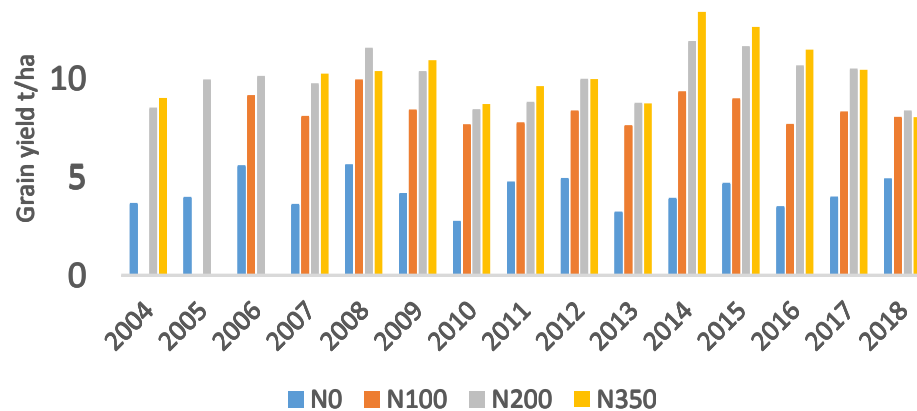
N100: 100:0
 N200:
 100:100
 N350:
 175:175

- Harvested 22/08/18

Soil Mineral N



Diversity Mean grain yields 2004-2018

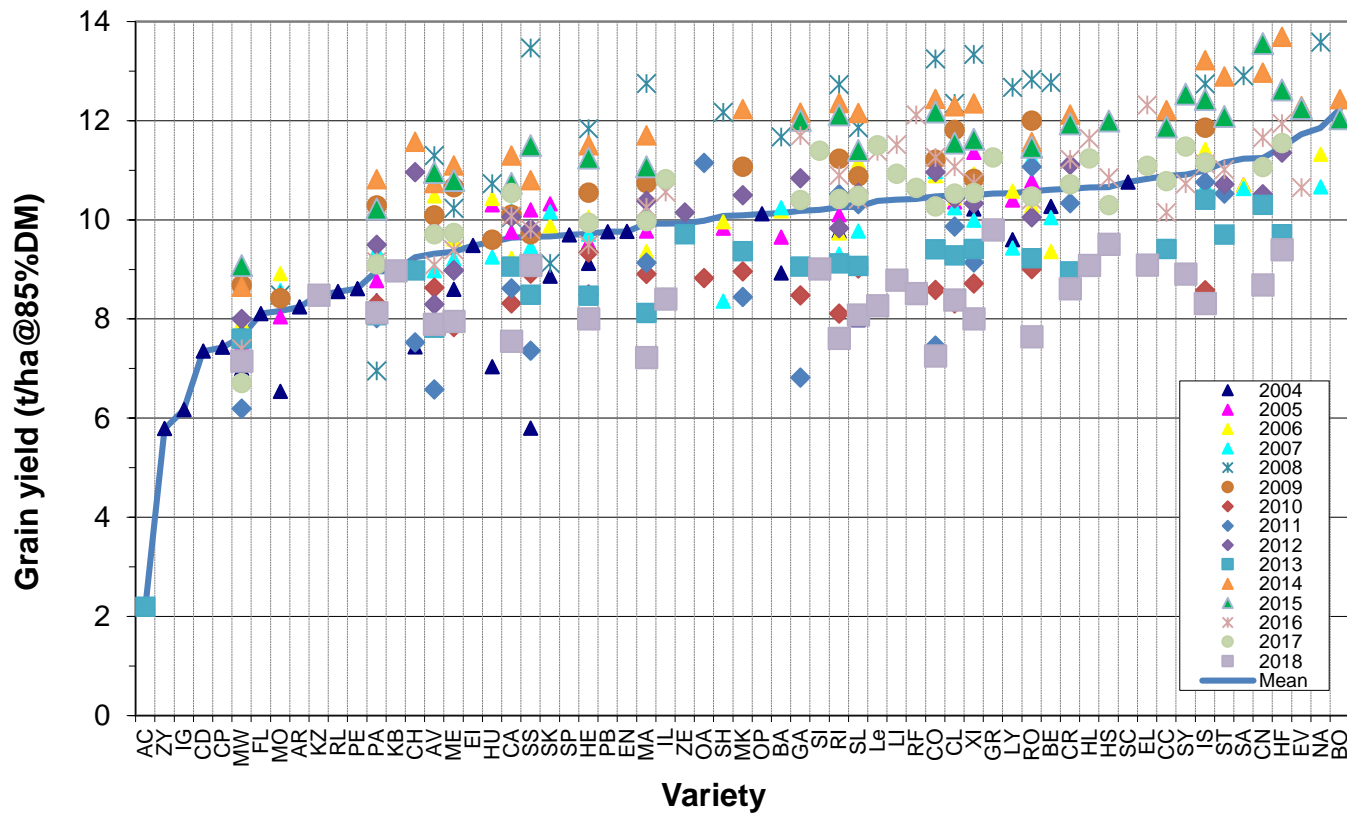


	2018 Yield	Rank	Mean
N0	4.91	4	4.22
N100	8.04	9	8.42
N200	8.37	15	9.95
N350	8.02	13	10.27

Year to year variation for selected varieties (N200)



N200 yields



2019 Varieties

Wheat varieties for WGIN/DFW NUE experiment
2018/19
16th year

W=WGIN data, D=desk study

NB lost Conq, Cordiale, Evol, Heref, Hystar, Illust, Leeds, Lilli, Reflect, Skyfall

Variety	Source	Nabim	Rationale	inclusion in trial requested by	Previous years of trials (harvest year)
1. Avalon		1	WGIN DH parent; Low NupE & NutE (D) WUE trial	PB, RG, MJH	05-18
2. Cadenza		2	WGIN DH parent; Best NupE (W) WUE trial	PB, RG, MJH	04-18
3. Claire	LIM	3	Was biggest area on RL; WGIN DH parent; Good second wheat	PB,PS	05-18
4. Crusoe	LIM	2	Carries dicoccoides. Shows the 'stay green' character		11-18
5. Graham	Syn	4	High yielding	MH	17-18
6. Hereward	RAGT	1	Best protein on RL; benchmark bread variety. BBSRC Quality project WUE trial	PB,PS	04-18
7. Hylux	Saaten Union		Hybrid. Early flowering and maturing. Can be mildew susceptible; treat T0. Good under stress?MH Breadmaking?		16-18
8. Istabraq	LIM	4	Best yield on RL; Distilling cultivar; In LINK 'GREENgrain'; Good second wheat. BBSRC Quality project. WUE trial	PB,PS	05-18
9. KWS Barrel	KWS	3	Claimed high yielding	MH, AR	18
10. KWS Zyatt	KWS	1	Claimed high yielding	MH, AR	18
11. Malacca	KWS	1	Biggest Group 1 area; DH choice; Low NupE, high NutE (W). BBSRC Quality project	PS	04-18
12. Maris Widgeon		1	Tall (rht), old cultivar WUE trial	PB, AM	04-18
13. Mercia		1	Low NupE & NutE (desk); Low Canopy N requirement; In IGF micro-array. WUE trial . RHT series	RG	04, 06-18
14. Paragon	RAGT	1	Spring variety; WGIN mutagenesis population; High NupE (W)	PB	04-18
15. Riband	RAGT	3	WGIN DH parent; Distilling cultivar; In LINK 'GREENgrain'; High NutE (W)	RG	04-18
16. Robigus	KWS	3	Best Group 3 yield; Best NUE, high NupE & NutE (D); Good second wheat. WUE trial	PB, AM	05-18
17. Siskin	KWS	2	Consistent across regions. In CINAG trial inc at NW	K Goulding	17-18
18. Soissons	Elsoms	2	WGIN DH parent; Early maturing; High NupE, low NutE (W) WUE trial	PB, RG, AM	04-18 (no 17 data)
19. Solstice	LIM	2	Biggest Group 2 area; DH choice; Worst NupE (W)	RG	04-18
20. Xi19	LIM	1	Best Group 1 yield; High NUE, NupE, NutE (D); Low NupE (W).	PB,PS	04-18



High Resolution imaging by UAV

Goal:

Achieve high resolution images to enable automated detection of traits not identifiable in low resolution images.

High resolution achieved by flying at lower altitude and/or with a longer focal length lens

Benefits

Avoids requirement to orthomosaic images

Issues

Images must be collected at precise & known location

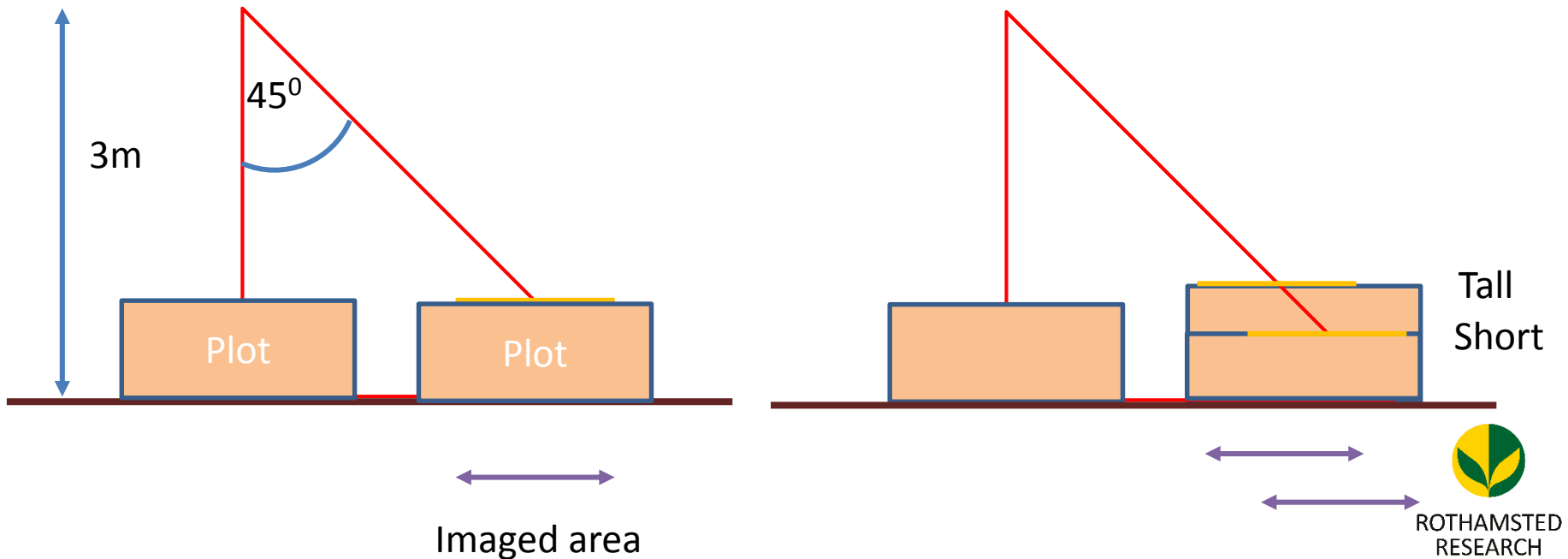
Low flight speed/and or fast shutter speed required to avoid blurr



Field of view

Altitude	FoV (m)	GSD (mm)	FoV @ 45 degrees
3m	1.15 x 0.87	0.22	1.15 x 1.23
5m	1.92 x 1.44	0.37	1.92 x 2.04

45mm lens, 45degree angle
(Scanalyser resolution 0.26mm)

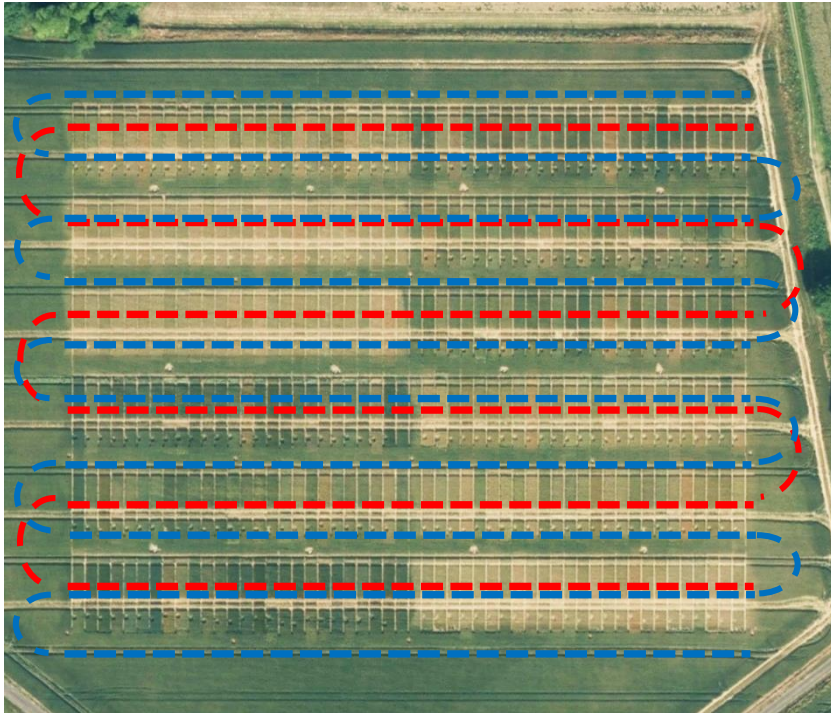


Downdraft

Here Andrew showed several short videos to highlight the increased downdraft when lowering flight height of the drone



Flight time



This experiment:

186m x 135m

Current flight pattern for mosaicking:

$$(186 * 10) + (135 * 2) = 2130\text{m}$$

$$\text{@}4\text{m/s} = 8.9\text{min}$$

Low altitude flight pattern:

$$(186 * 6) + (135 * 2) = 1386\text{m}$$

$$\text{@}2\text{m/s} = 11.6\text{ min}$$



Anthesis detection

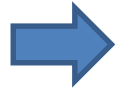


16M pixels, f/2.2, 1/2700sec, ISO100, 0 stop EV. 45mm lens.
Camera @ 45 degrees (Static images)



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RESEARCH

Image quality



3m altitude. 16M pixels, f/13, 1/1000sec, ISO100, -1 stop
EV. 45mm lens. 2 m/s flight speed



ROTHAMSTED
RESEARCH

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ROTHAMSTED
RESEARCH



Farm Staff

Stephen Goward

Chris Mackay

Nick Chichester-Miles



Department
for Environment
Food & Rural Affairs



Resilience to foliar and root fungal pathogens

Vanessa McMillan



Department
for Environment
Food & Rural Affairs

WGIN 4 objectives



ROTHAMSTED
RESEARCH

- **Resistance to septoria leaf blotch**
- Resistance to yellow rust
- 3N ancestral introgression rooting trait
- Resistance to take-all disease in *Triticum monococcum*
- *Mlo* mediated resistance to powdery mildew

Septoria leaf blotch



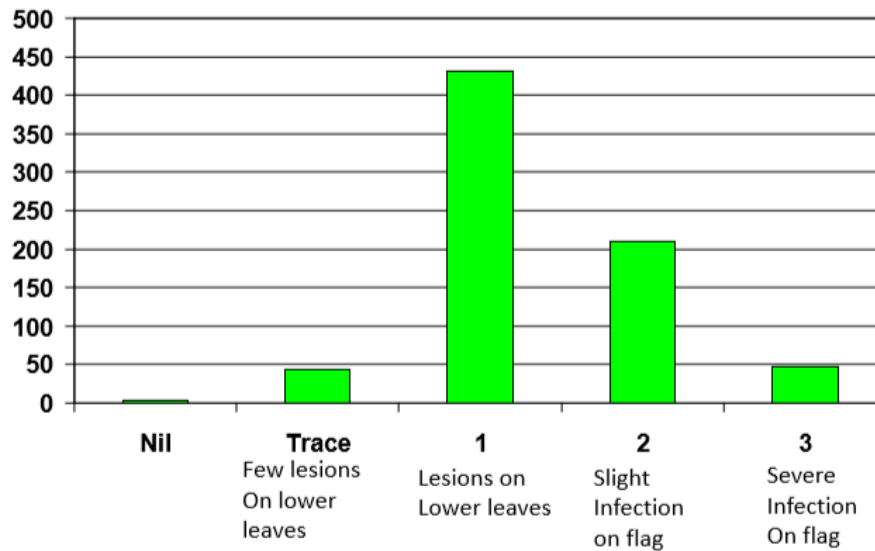
ROTHAMSTED
RESEARCH

Aim: To identify novel sources of resistance against Septoria which are robustly expressed across multiple field seasons

Germplasm

- Watkins wheat landraces (trace or no Septoria in 2008 field trial, n=50)
- CIMMYT Septoria Observation Nursery (n=50)
- Wheat genotypes with known *Stb* resistance genes (n=15)

Watkins field trial 2008



Genotype	R gene/reason for including in trial
Sullivan	Stb1
Bulgaria 88	Stb1 + Stb6
Israel 493	Stb3 + Stb6
Tadinia	Stb4 + Stb6
Synthetic 6x	Stb5
Felder	Stb6?
Estanzuela Federal	Stb7
Arina	Stb6 + Stb15
Courtot	Stb9
Kavkaz K4500 L.6.A.4	Stb10 + Stb12 + Stb7 + Stb6
TE 9111	Stb11 + Stb7 + Stb6
Salamouni	Stb13 + Stb14 + Stb6
Synthetic M3 (W7976)	Stb16q + Stb17
Balance	Stb18 + Stb6
Stb16q	Stb16q

Septoria Field Trial Design



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- Two replicates per treatment (limited seed availability)
- Outer plot rows sown to Avalon (highly susceptible to Septoria)
- Natural infection
- Rust specific fungicide regime

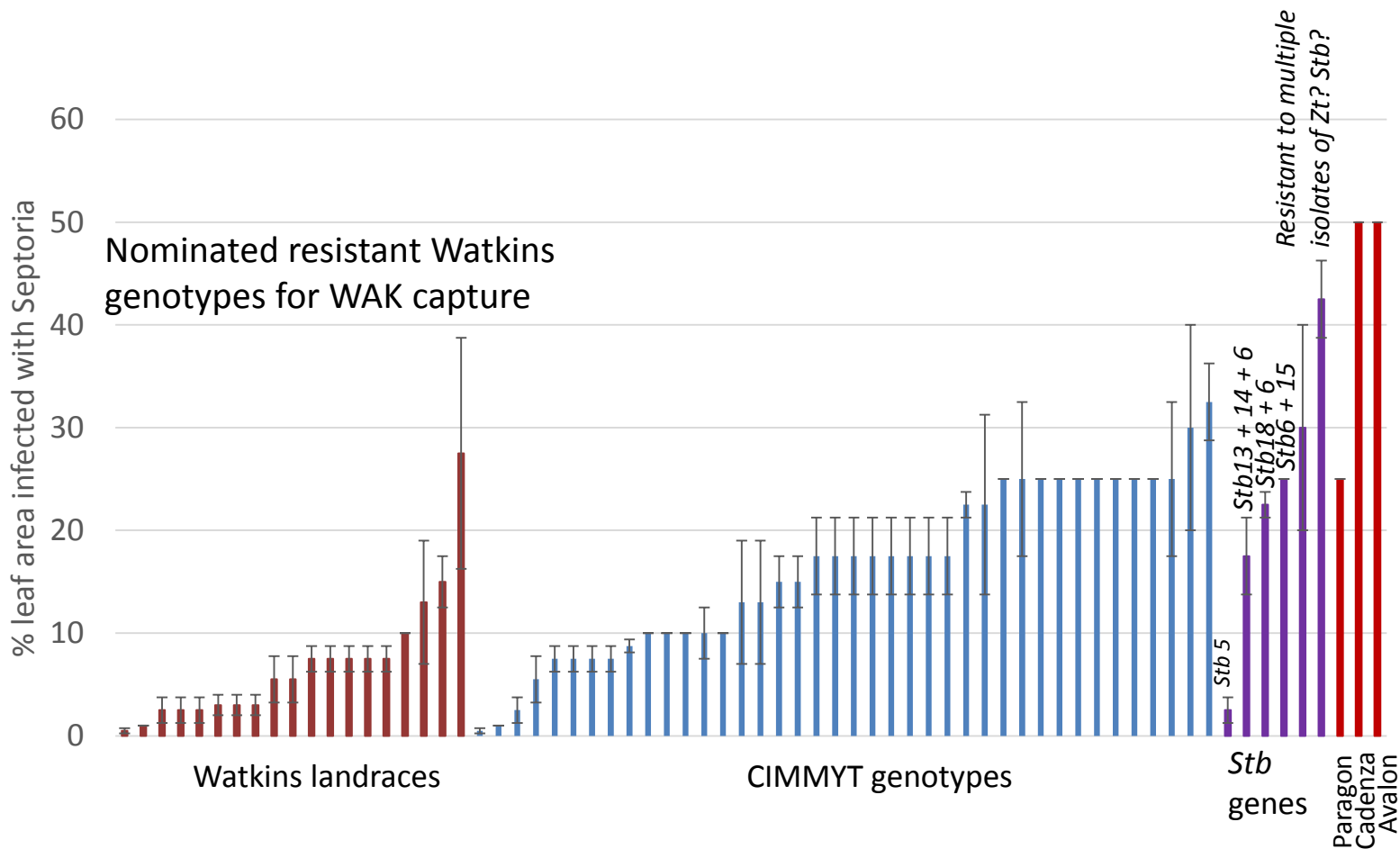
- Hertfordshire = relatively low disease pressure area for Septoria
artificially inoculate 2018/19 field trial with mixture of Zt isolates
- Low humidity (low plant density and relatively large path areas)
increase seed rate and plot size in 2018/19 field trial
- Dry weather in late spring and early summer, no irrigation possible
trial to be irrigated in 2018/19 using new equipment purchased by
RRes Farm
- T0 spray missed due to wet weather conditions, majority of
genotypes highly susceptible to yellow rust increase number of
sprays in 2018/19, consult with RRes agronomist on latest fungicide
advice

Variation in susceptibility to Zt



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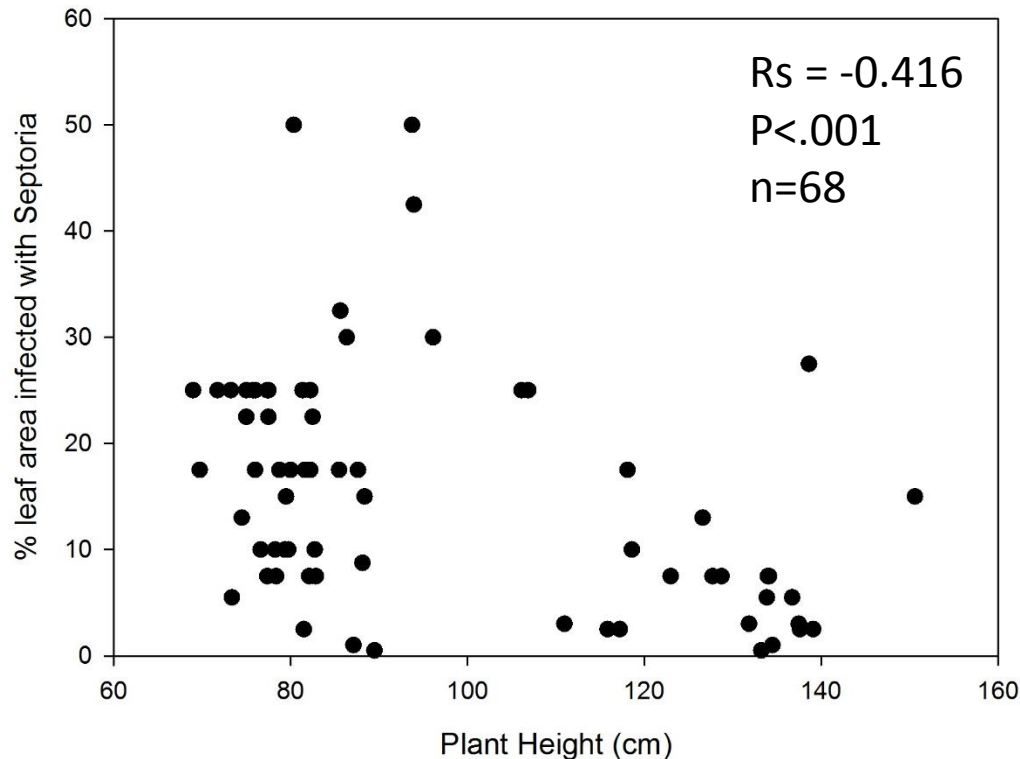
Leaf 3 assessments 8th June 2018



Relationship between plant height and disease severity



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Moderate negative correlation between plant height and % septoria infection

Some genotypes may be displaying disease escape phenotypes

Repeat field trial to be drilled October 2018

WGIN 4 objectives



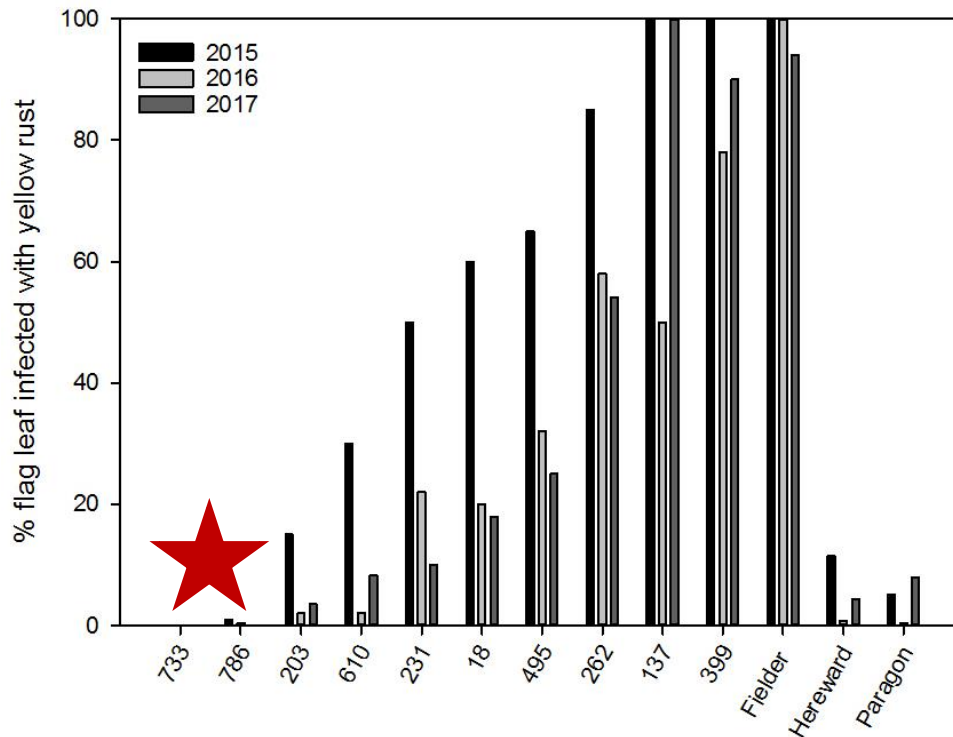
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RESEARCH

- Resistance to septoria leaf blotch
- **Resistance to yellow rust**
- 3N ancestral introgression rooting trait
- Resistance to take-all disease in *Triticum monococcum*
- *Mlo* mediated resistance to powdery mildew

Evidence of resistance to yellow rust



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Adult plant resistance in field trials

- Five Watkins genotypes with moderate/strong resistance against yellow rust (WGIN 3)



WGIN4 = F3 bulked segregant analysis on two most resistant Watkins genotypes

2018/19 Field Trial

F2 and F3 families will be phenotyped to identify homozygous susceptible and resistant lines

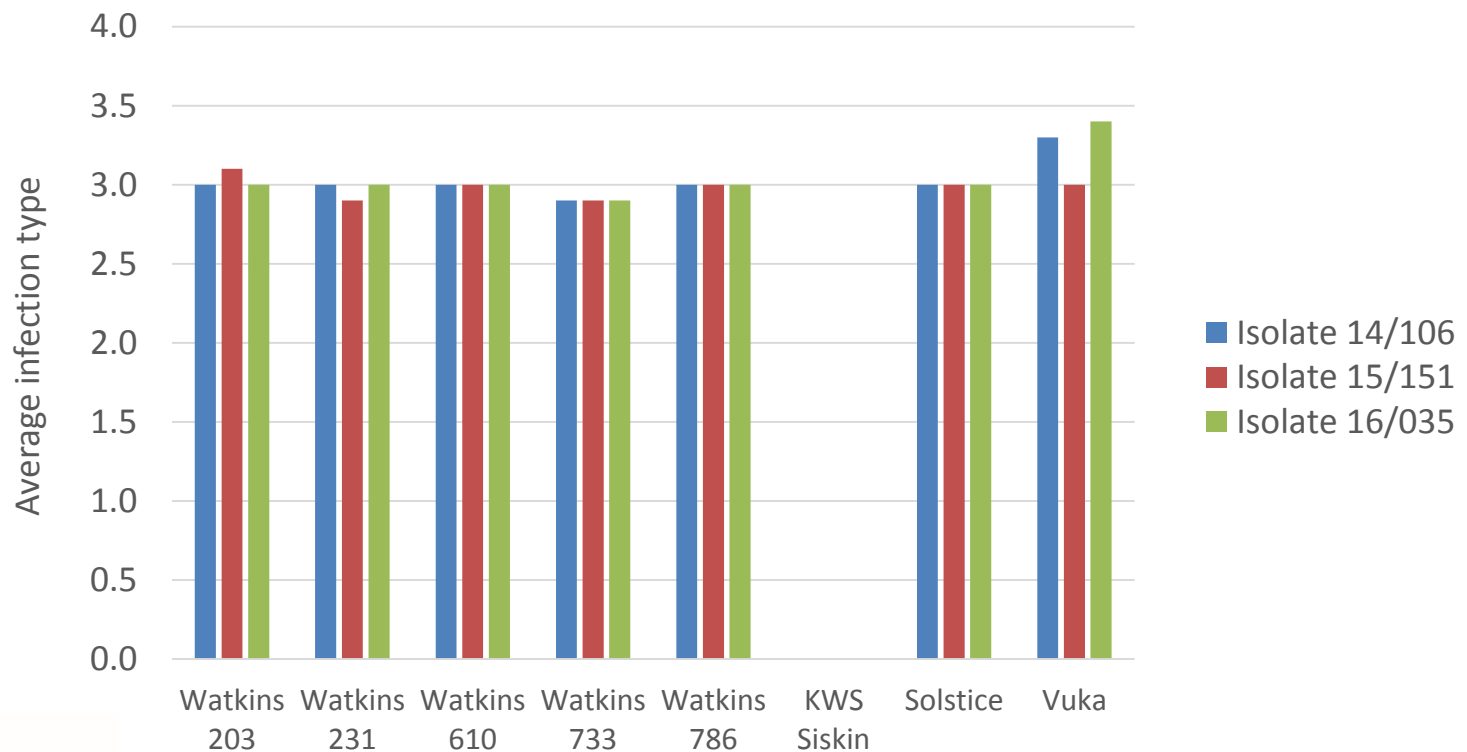
Seedling virulence testing @ NIAB



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- 14/106 Purple 3 (previously known as the Kranich race)
- 15/151 Blue 7 (previously known as the Invicta race)
- 16/035 Red 24 (new in 2016, caused disease on Britannia, Myriad, Zulu, Reflection amongst others)

All Watkins genotypes are susceptible at the seedling stage



■ Isolate 14/106
■ Isolate 15/151
■ Isolate 16/035

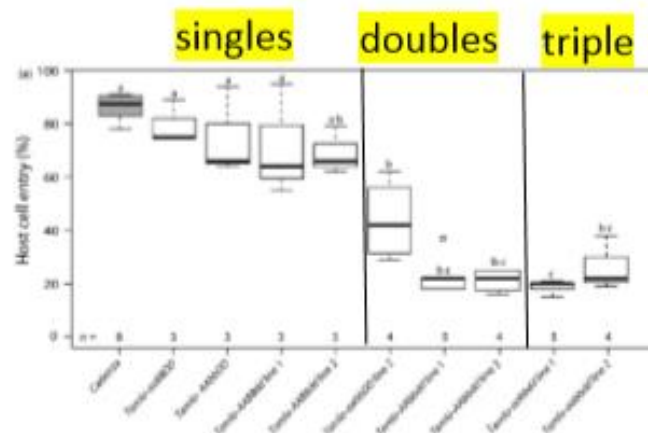
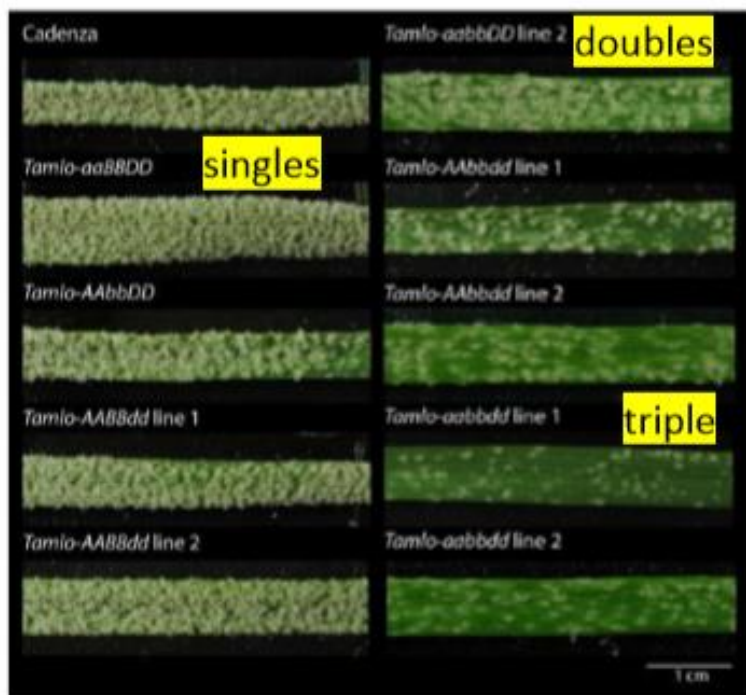
WGIN 4 objectives



ROTHAMSTED
RESEARCH

- Resistance to septoria leaf blotch
- Resistance to yellow rust
- 3N ancestral introgression rooting trait
- Resistance to take-all disease in *Triticum monococcum*
- ***Mlo* mediated resistance to powdery mildew**

TILLING wheat for *mlo* mediated mildew resistance



Seedling
glasshouse
screen

Cv Cadenza

Initially WGIN
funded

Plant Biotechnology
Journal

2017

aab SEB
Wheat and Barley Research Society

Plant Biotechnology Journal (2017) 15, pp. 367–376

doi: 10.1111/tpb.12691

mlo-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach

Johanna Acevedo-García¹, David Spencer¹, Hannah Theron¹, Anja Reinstädler¹, Kim Hammond-Kosack², Andrew L. Phillips² and Ralph Panstruga^{1*}

Department
for Environment
Food & Rural Affairs

Funders: German Federal Ministry of
Food and Agriculture

Germany Society for the
Advancement of Plant Innovation

Are there trade offs under field conditions?



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RESEARCH

2017/18 Spring sown multiplication field trial

- 18 selected double and triple mutants (single replicate, 30 seeds)
- Replicated plots of Cadenza wildtype
- Foliar disease observations
- Ear emergence and plant heights

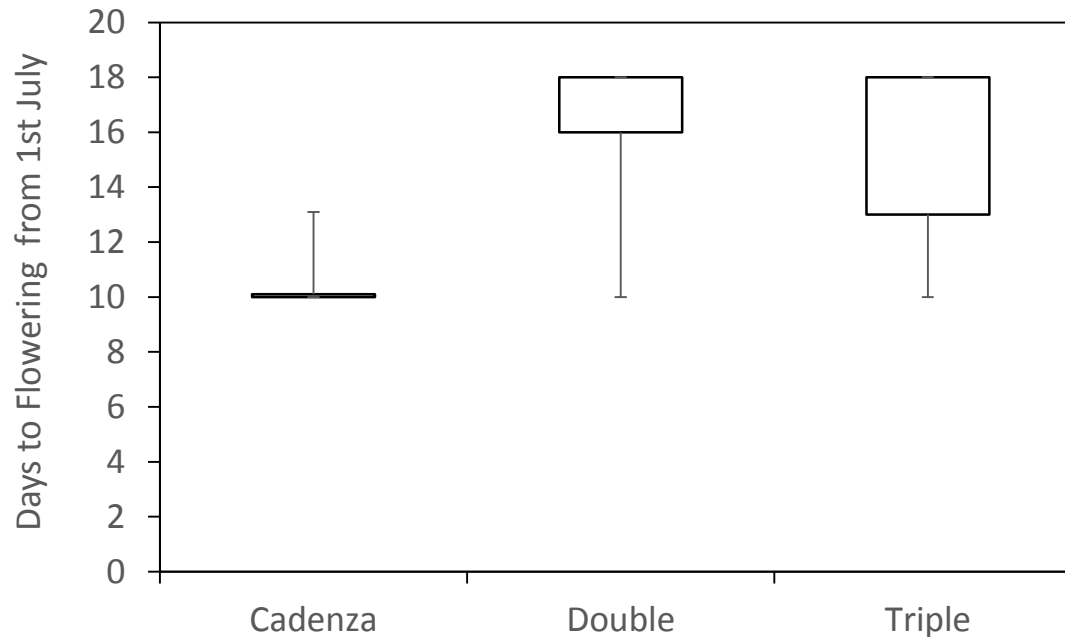


Days to Flowering



ROTHAMSTED
RESEARCH

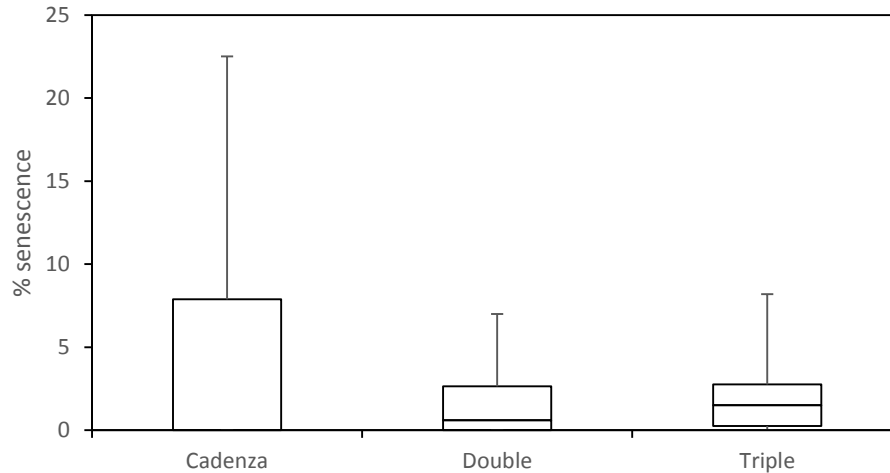
6 Cadenza plots, 4 double mutants, 14 triple mutants



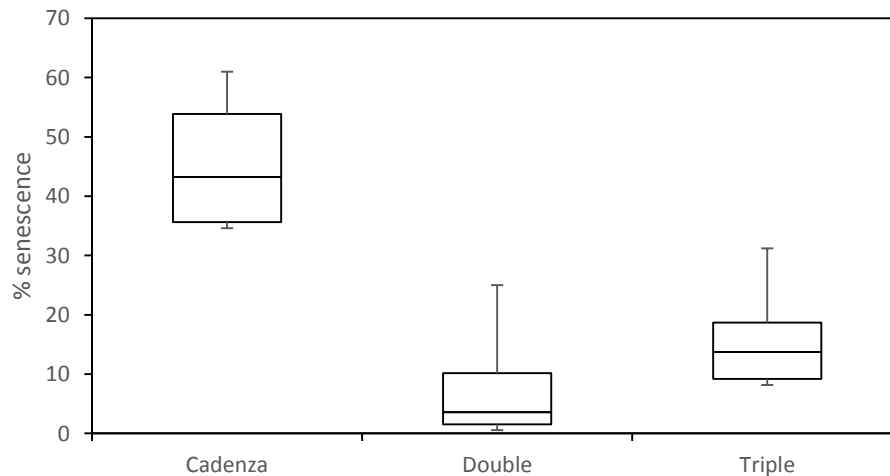
Many genotypes started flowering before full ear emergence due to drought conditions

Cadenza = earlier flowering than double or triple mutants

Flag leaf



Leaf 2



Cadenza plots much more senesced...related to earlier ear emergence?

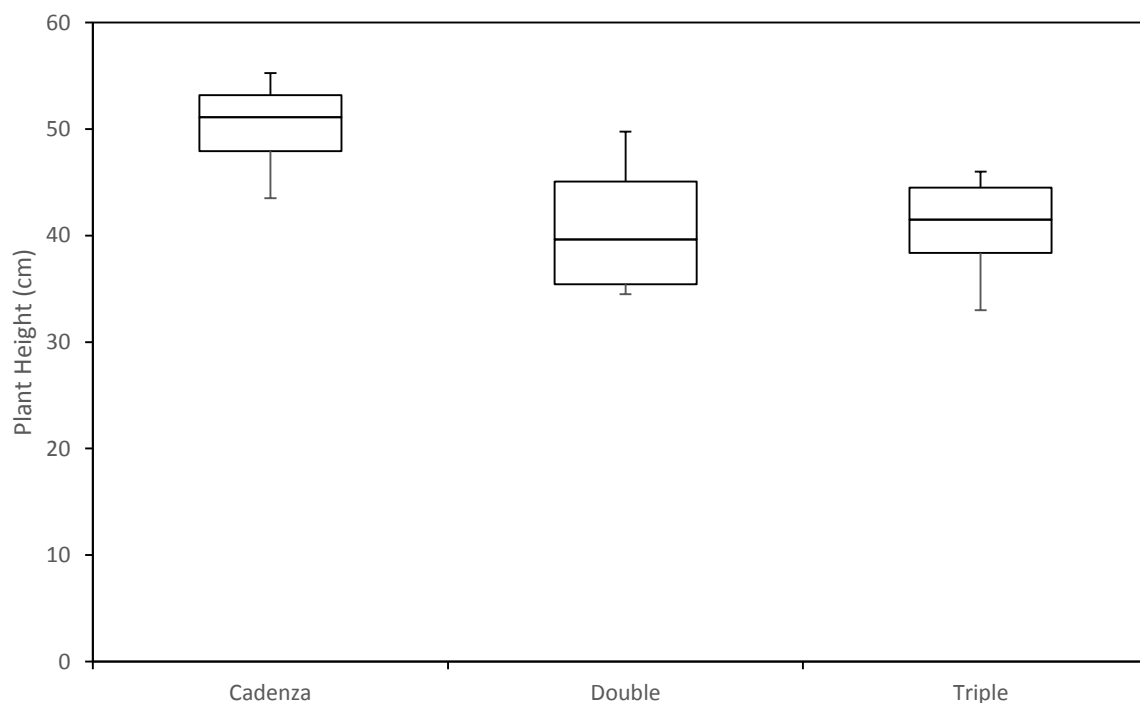
Impact of drought?

Plant Height



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6 Cadenza plots, 4 double mutants, 14 triple mutants



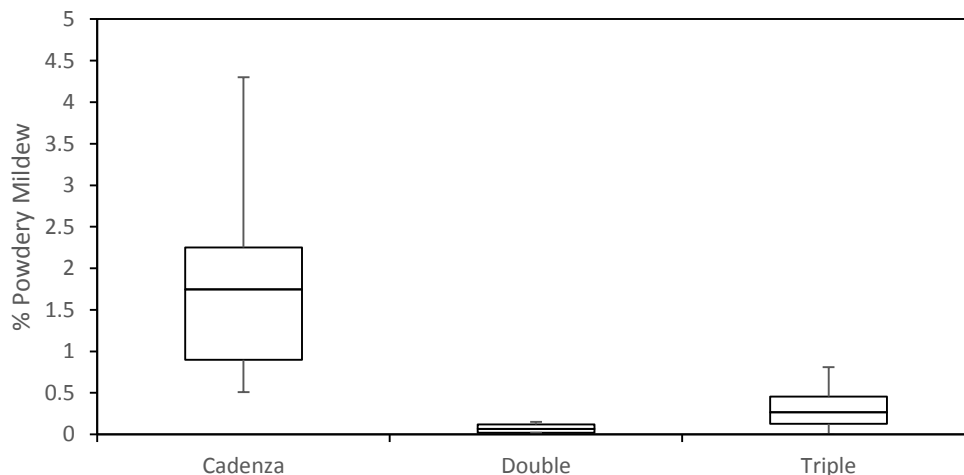
Wildtype Cadenza slightly taller?

Powdery Mildew Assessments



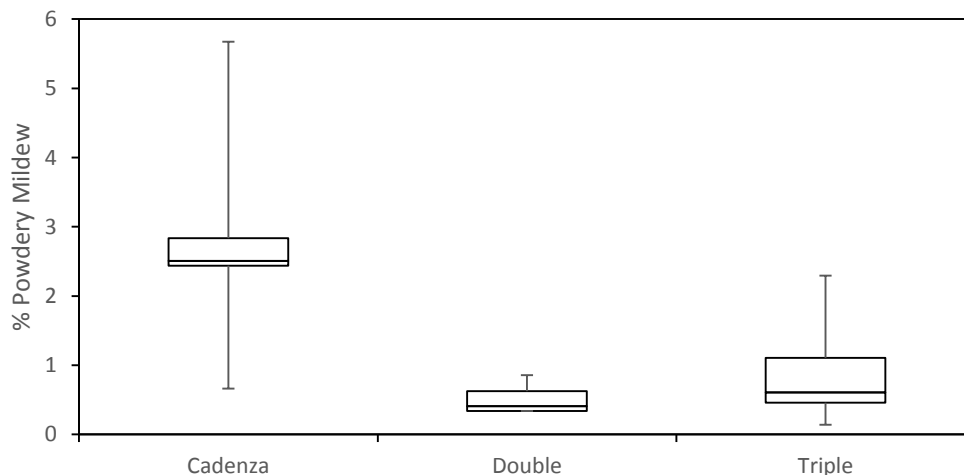
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Flag Leaf



Low powdery mildew disease pressure

Leaf 2



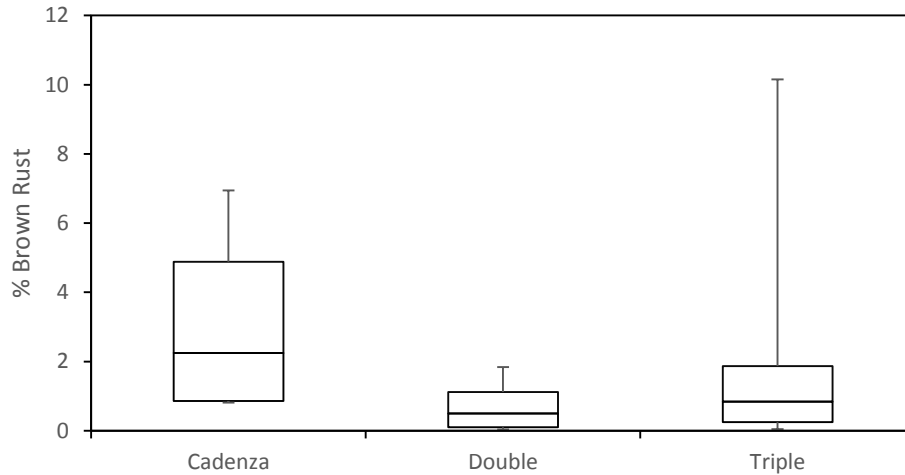
Trend for a reduction in powdery mildew infection for double and triple mutants

Brown Rust Assessments



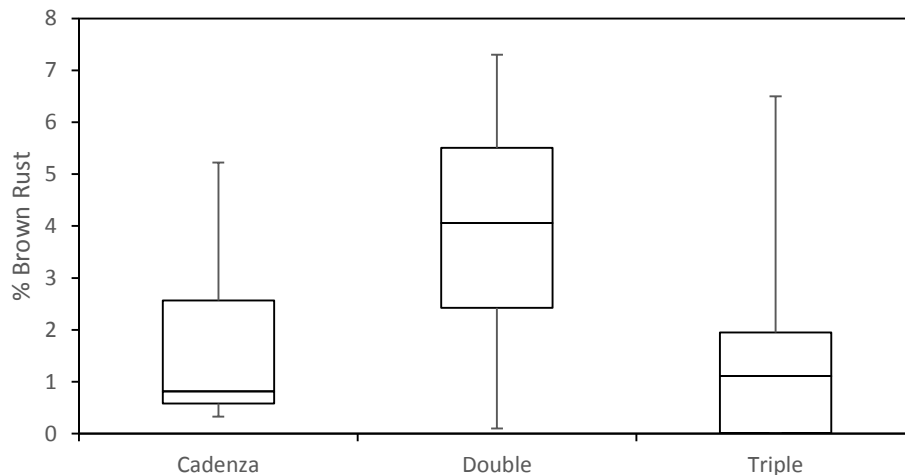
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Flag leaf



Overall relatively low brown rust disease levels across field trial

Leaf 2



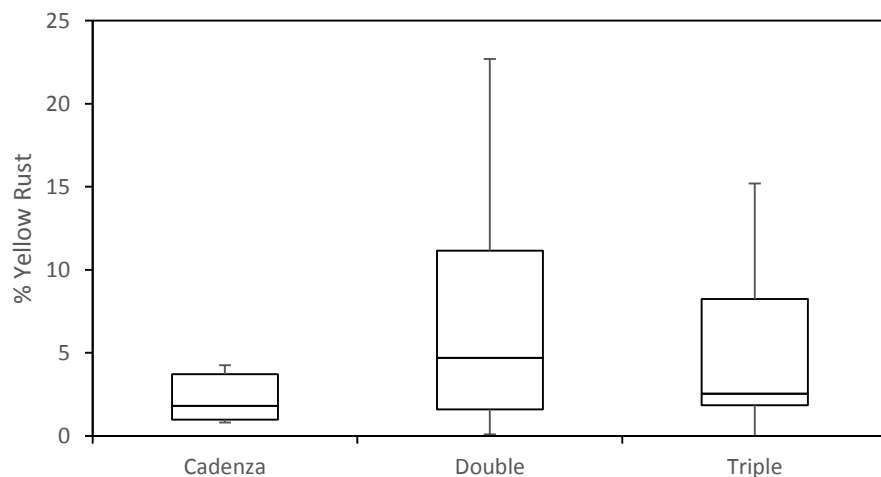
A couple of triple mutants with relatively high brown rust scores – perhaps due to location in trial?

Yellow Rust Assessments

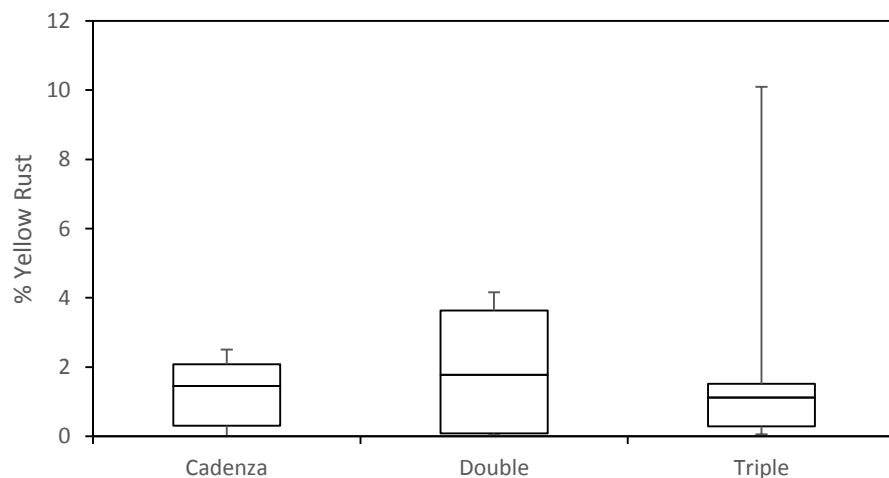


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Flag Leaf



Leaf 2



Some doubles and triples with higher yellow rust infection

***Mlo* mediated resistance**



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2019 and 2020

Replicated field trials to explore the double and triple lines susceptibility/resistance to additional pathogens (as a winter crop)
– yellow rust, brown rust, septoria and fusarium

Many thanks to



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Jessica Hammond (Plant Pathology Apprentice)

Summer students

Erin Baggs (2015)
Eleanor Leane (2015)
Tessa Reid (2015)
Laurie Neal (2015,2016&2017)
Alex Chambers-Ostler (2016)
Leanne Freeman (2016,2017&2018)
Jamie Hawkesford (2017)
Ellen Farnham (2017&2018)
Georgie Halford (2018)
Eoin Canning (2018)
Niamh Kavanagh (2018)

Rodger White and Stephen Powers - statistics

RRes Farm and glasshouse staff



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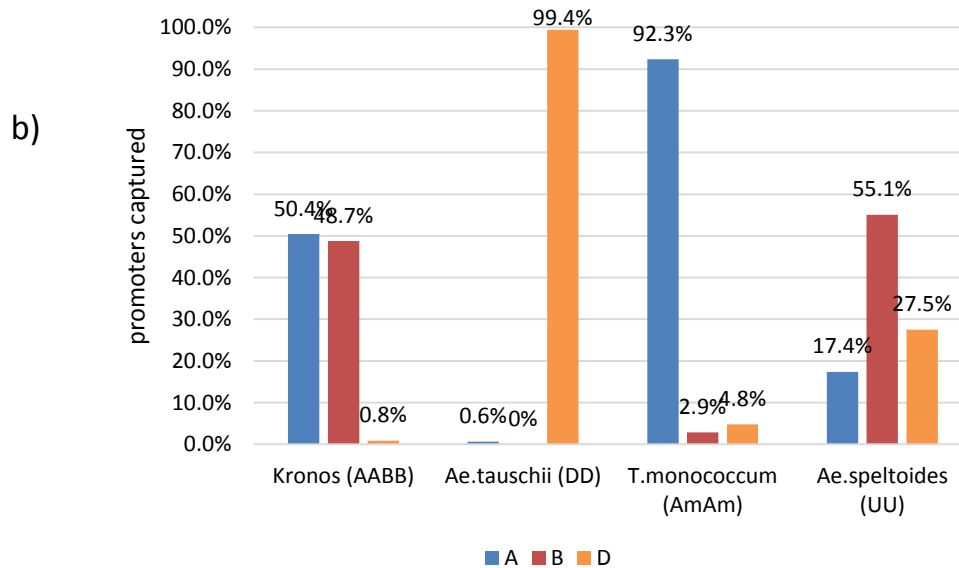
Update on Promotome Capture

Michael Hammond-Kosack (WGIN PA)

WGIN MM October 4th 2018 @ Limagrain

a)

homoeologue	A				B				D			
cultivar	KR	ENT	Tm	ASP	KR	ENT	Tm	ASP	KR	ENT	Tm	ASP
Sequencing coverage	+	-	+	-	+	-	-	+	-	+	-	-

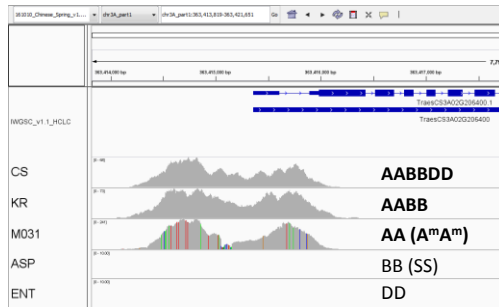


homoeologue specific MYbaits capture a) Promotome capture is homoeologue specific only if these coverage patterns are observed. b) observed coverage patterns. Please note that while Kronos and Ae. tauschii have the exact AB and D subgenomes, respectively, T.monococcum and Ae.speltoides are only related to the A and B subgenomes, respectively.

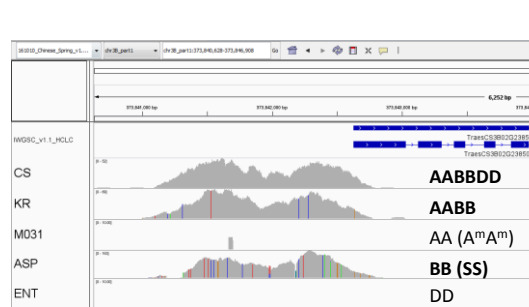
A working example of the type of results + specificity of the probes for A, B and D genomes

T4-57 (AK366042, Barley_2012_AC3_genes_3H 135.482)

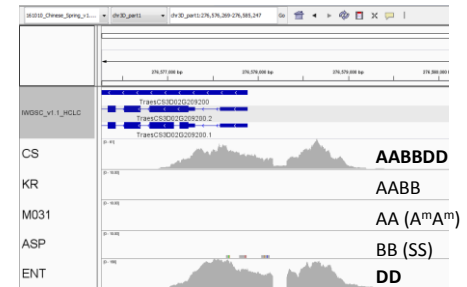
A



B

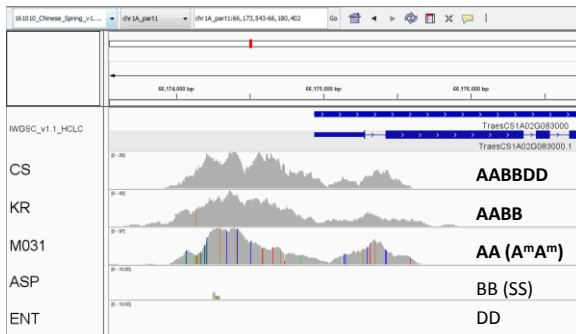


D



T1-20 (APG/OsPIL16) A

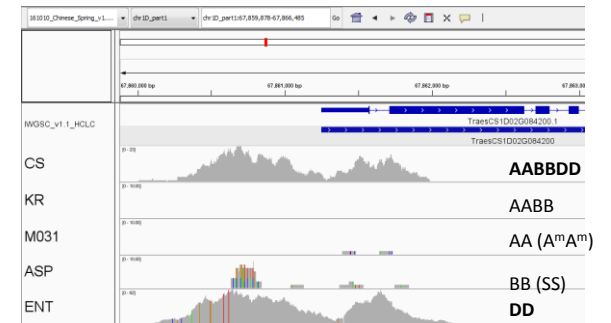
A



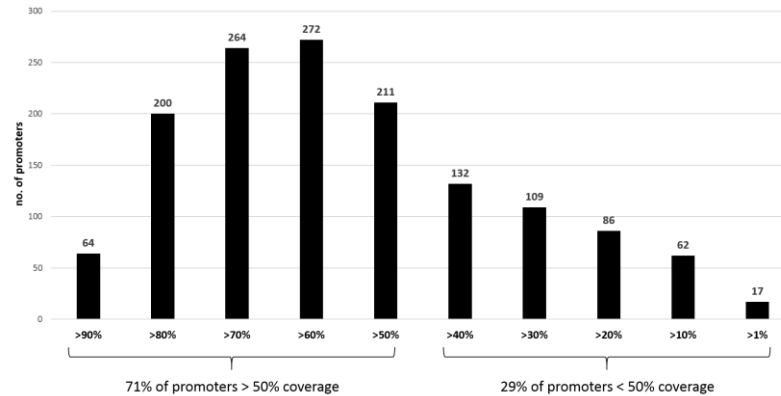
B



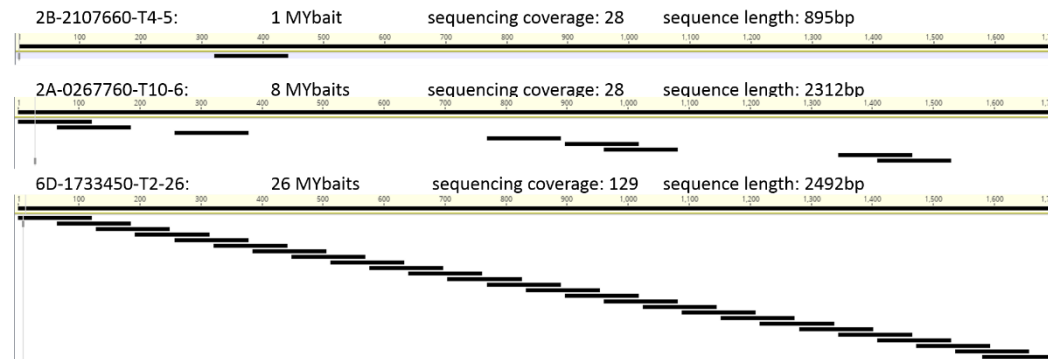
D



a)

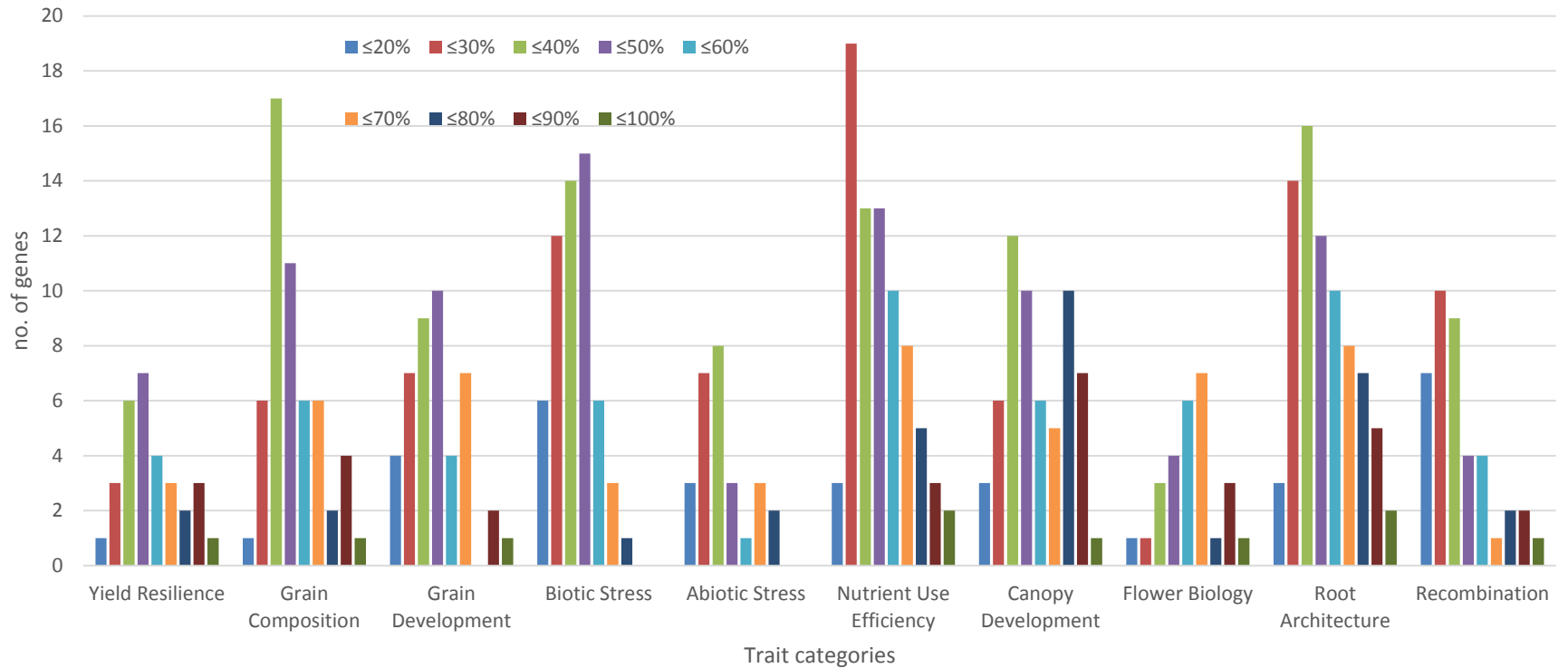


b)

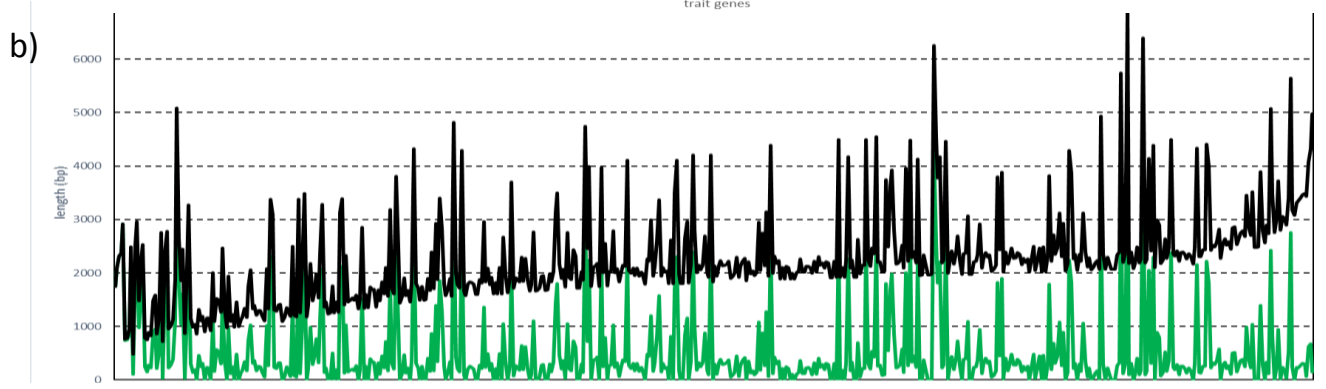
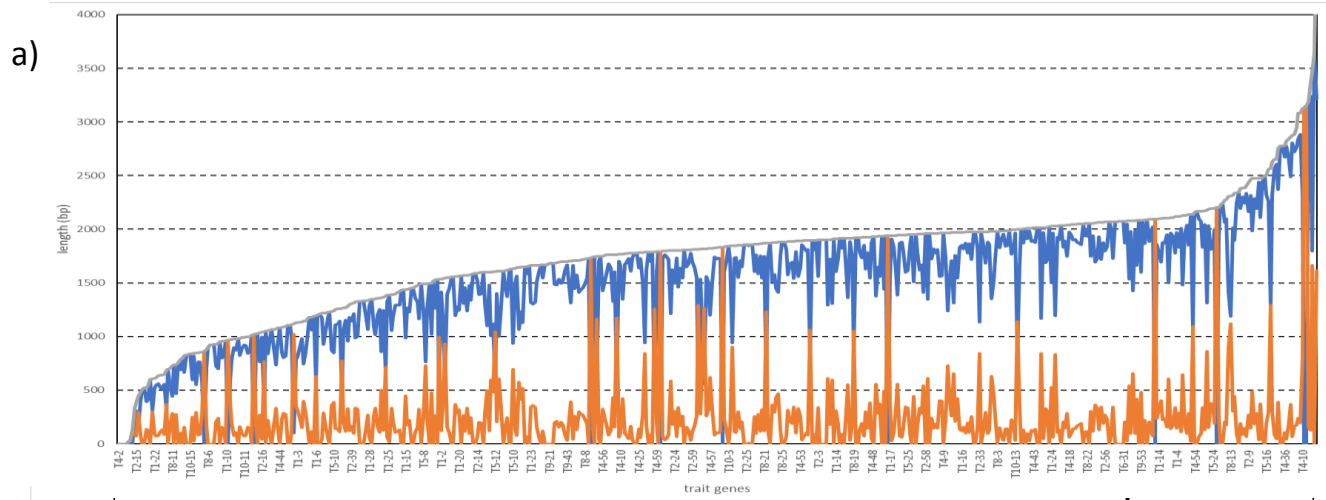


MYbaits promoter cover under the high stringency conditions used

- a) Promoters grouped by percentage of sequence cover: any promoters with >50% MYbaits cover would be expected to yield the target sequence length (1700bp);
- b) examples of lowest (T4-5), medium (T10-6) and highest cover possible (T2-26) showing the number and position (black bars) of MYbaits, sequencing coverage observed and sequence length obtained

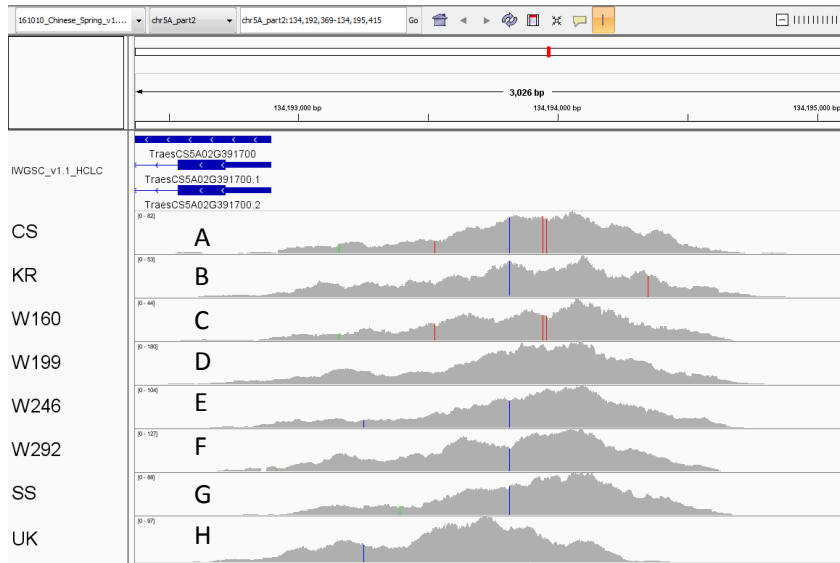


similarity of homoeologue promoter (& 5'UTR) sequences (1700bp) for each trait category, grouped by increasing percentage of similarity.



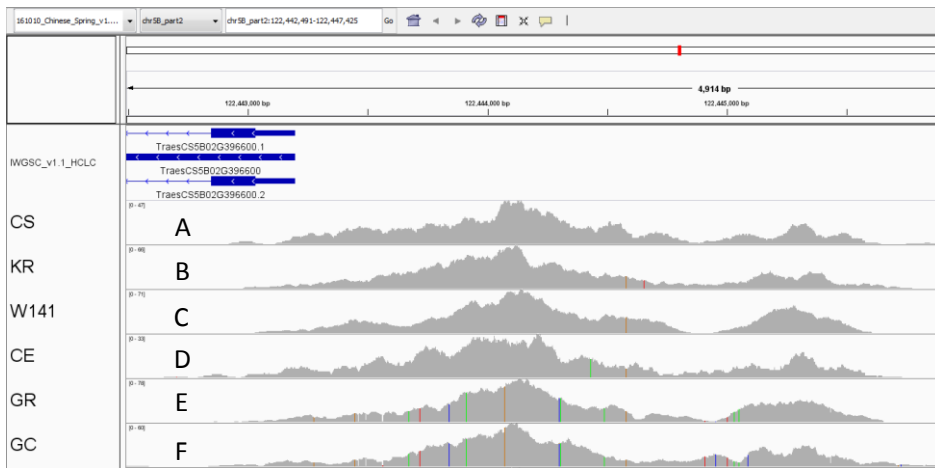
lengths of sequence captured for 602 trait genes. Genes are ordered by size of combined promoter and 5'UTR length. a) blue = promoter sequence, orange = 5'UTR, grey = promoter + 5'UTR; b) green = Exon/Intron sequence, black = total length of sequence captured for each gene. Nb: the X-axis contains all 602 genes analysed so far but only a few tags are shown for visibility's sake

VRN1 (T7-37) A homoeologue haplotypes



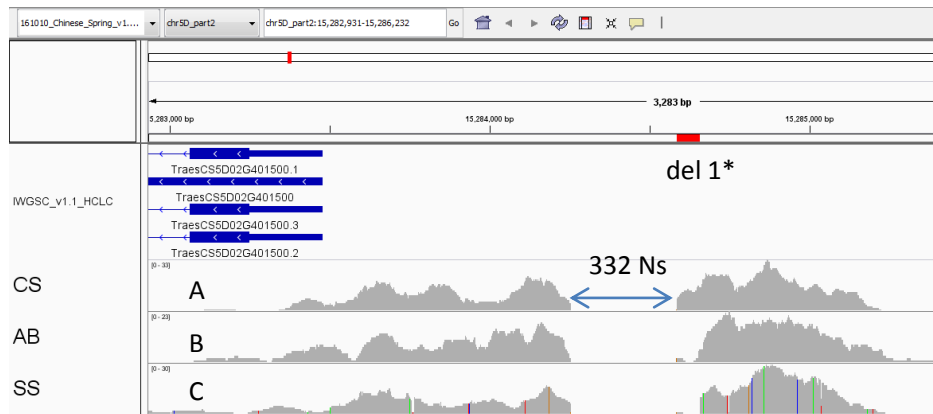
haplotype	SNPs	No.	cultivars
A	5	12	CS, W115, W141, W203, W209, W579, W777, BW, CA, PA, TA, XI
B	2	1	KR
C	4	2	W160, BW,
D	0	10	W199, CE, CL, CG, IQ, RO, SP, SU, AP, VE
E	2	53	W246, W387, W624, W733, AB, AL, AM, AV, BA, BR, BU, CH, CO, CR, DI, EI, FI, FL, GA, GC, GT, GL, GR, HF, HW, HO, HU, IS, JB, KSA, KSL, KSI, KTR, MA, MH, MW, MK, ME, NA, OA, PI, RE, RL, RI, RB, SA, SF, SO, SL, ST, VA, YU, ZE
F	1	3	W292, CP, RV,
G	2	1	SS
H	1	1	UK

VRN1 (T7-37) B homoeologue haplotypes



haplotype	SNPs	No.	cultivars
A	0	3	CS, W199, PI
B	2	5	KR, W387, AV, MW, RL,
C	1	70	W141, W160, W203, W209, W246, W292, W579, W624, W733, W777, W786, AB, AL, AM, BA, BW, BR, BU, CA, CH, CL, CP, CO, CG, CR, DI, EI, FI, FL, GA, GT, GL, HF, HW, HO, HU, IS, IQ, JB, KSA, KSL, KSI, KTR, MA, MH, MK, ME, NA, OA, PA, RE, RV, RI, RB, RO, SA, SC, SF, SL, SP, ST, SU, TA, UK, AP, VA, VE, XI, YU, ZE
D	2	2	CE, SO
E	20	2	GR, SS
F	21	2	GC, W115

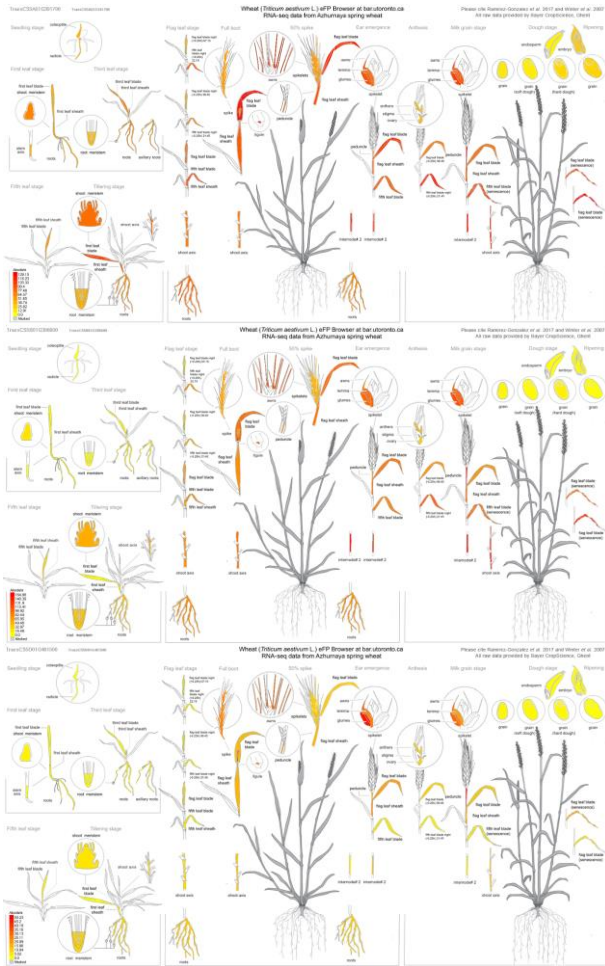
VRN1 (T7-37) D homoeologue haplotypes



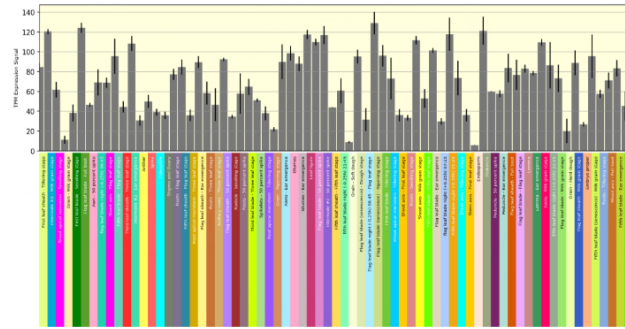
haplotype	SNPs	No.	cultivars
A	0	17	CS, W141, W246, W292, W777, BW, BR, BU, CA, CO, GA, KTR, OA, RO, AP, VE, YU
B	0, del1	65	W115, W160, W199, W203, W209, W387, W579, W624, W733, W786, AB, AL, AM, AV, BA, CE, CH, CL, CP, CG, CR, DI, EI, FI, FL, GC, GT, GL, GR, HF, HW, HO, HU, IS, IQ, JB, KSA, KSL, KSI, MA, MH, MW, MK, ME, NA, PA, PI, RE, RL, RV, RI, RB, SA, SC, SF, SO, SL, SP, ST, SU, TA, UK, VA, XI, ZE
C	15, del1	1	SS

*del1 = CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTA

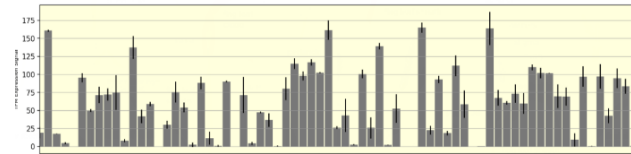
T7-37 (Vrn1) homoeologue expression



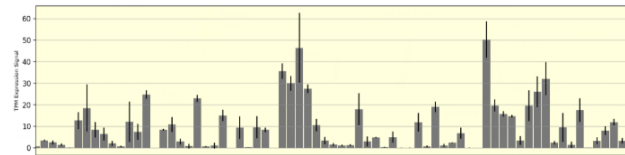
A



B



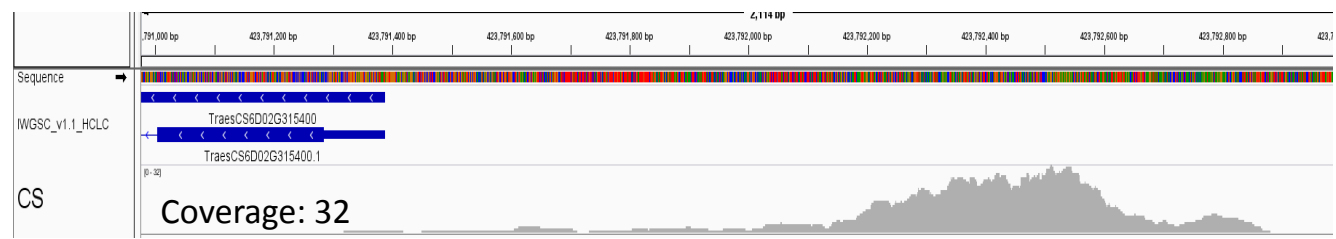
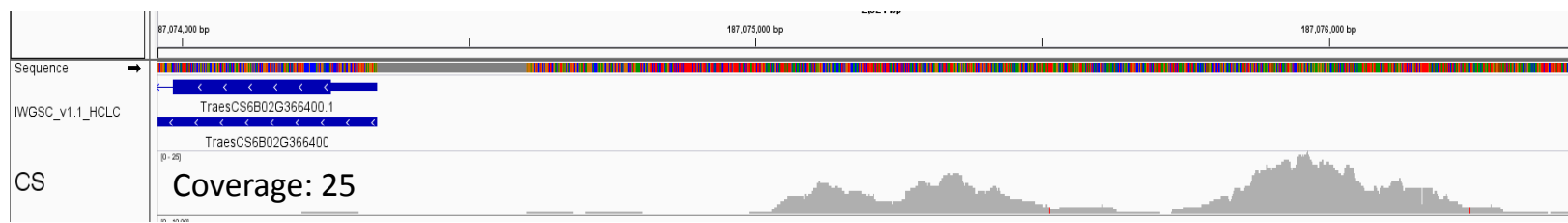
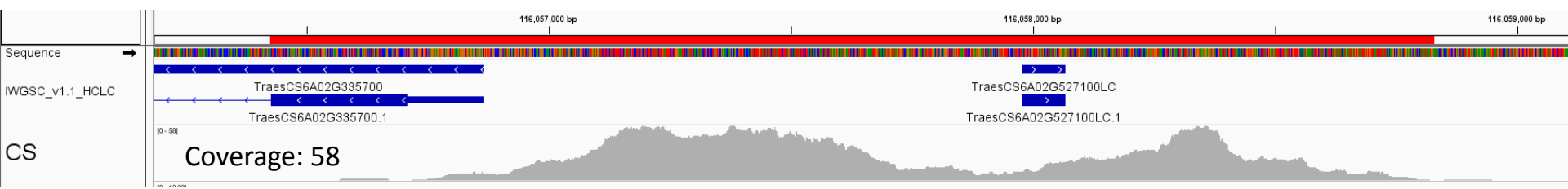
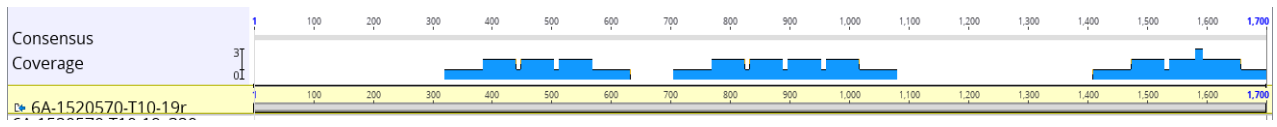
D

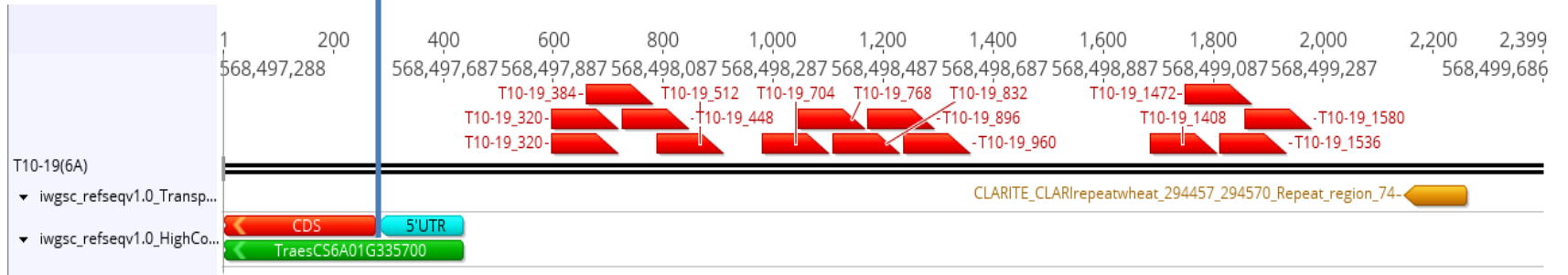
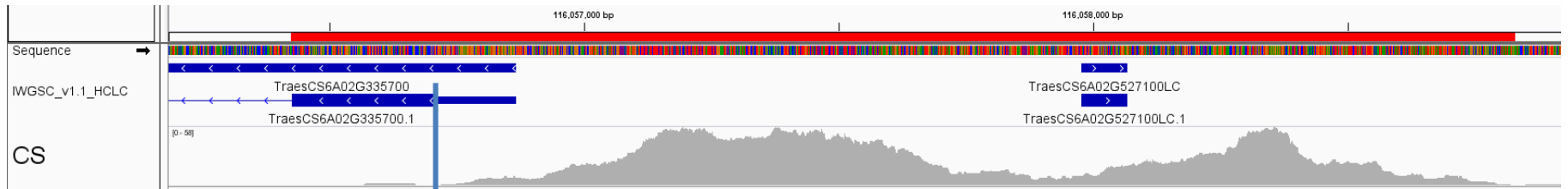


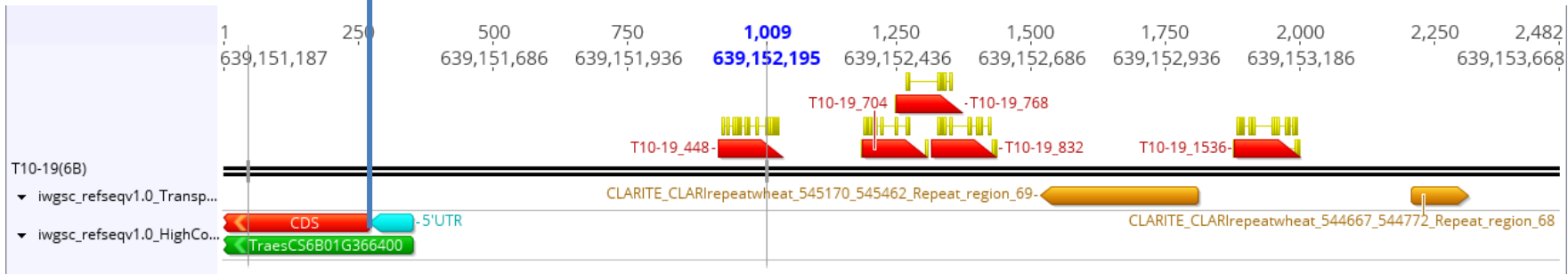
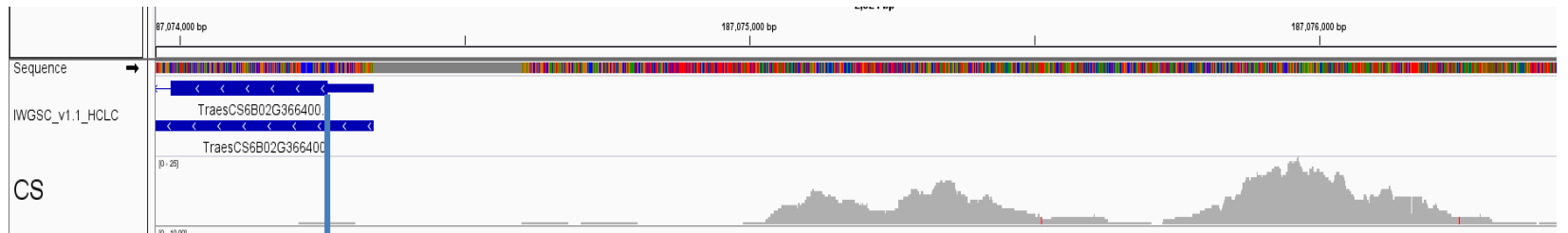
Subsequent homoeologues captured - how?

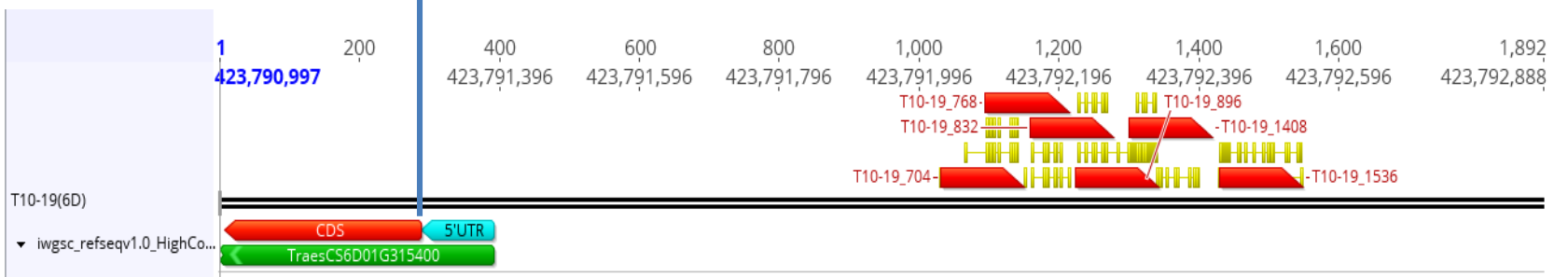
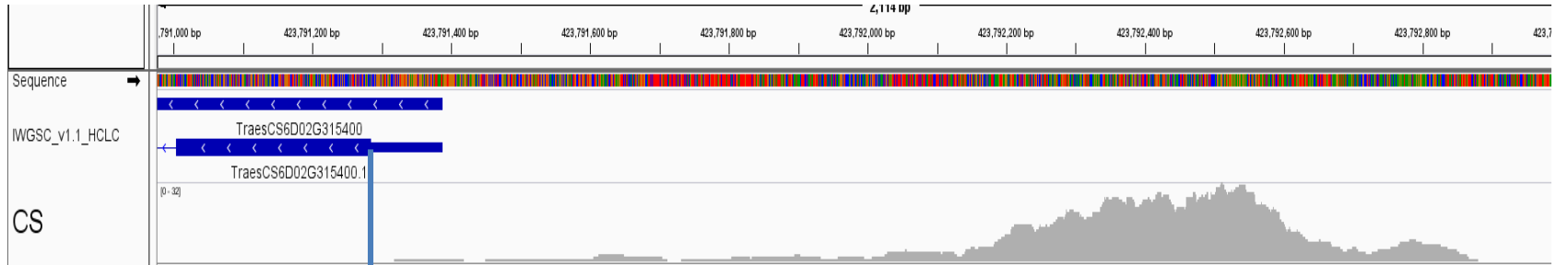
1. For **108 WGIN trait genes** only 1 or 2 homoeologue IDs were received
2. For **78** of these, the missing homoeologues can now be identified via the Wheat eFP Browser resulting in **101 subsequent homoeologues**
3. Of these, **83 have been captured** in the WGIN promotome experiment, despite the fact that **NO Mybaits** were synthesised for these promoters:
 - all observe the subgenome specificity, eg the subsequent D homoeologue only shows coverage for Ae.tauschii but not Kronos, T.monococcum or Ae.speltoides
 - Virtually all have **reduced coverage** (= no. of aligned sequenced fragments) and **shorter length**

T10-19 (RPA1a): only A homoeologue ID received, and Mybaits were only made for the A homoeologue promoter











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Update on *Triticum monococcum* Introgression

Michael Hammond-Kosack (WGIN PA)

WGIN MM October 4th 2018 @ Limagrain

Latest crossing strategy using tetraploid wheat as a bridging species

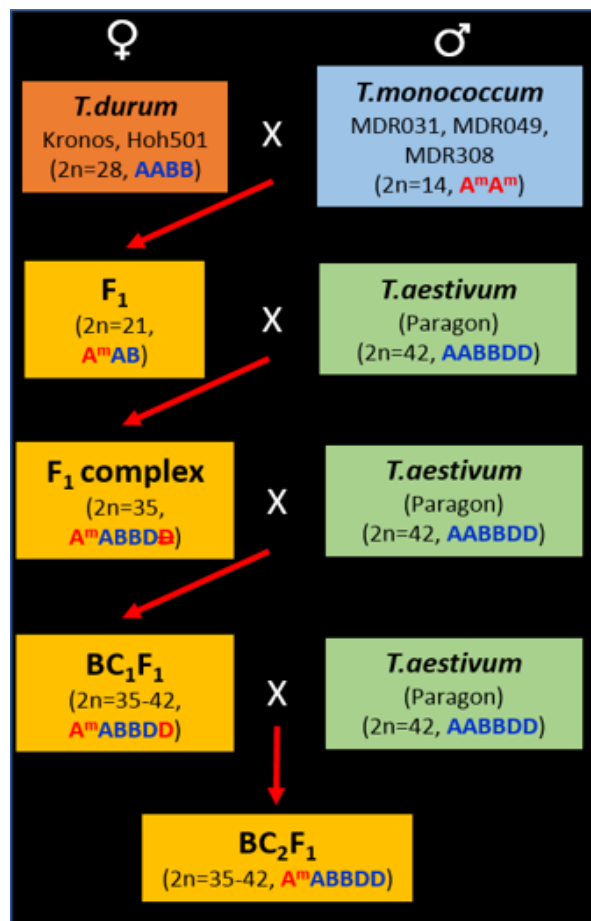
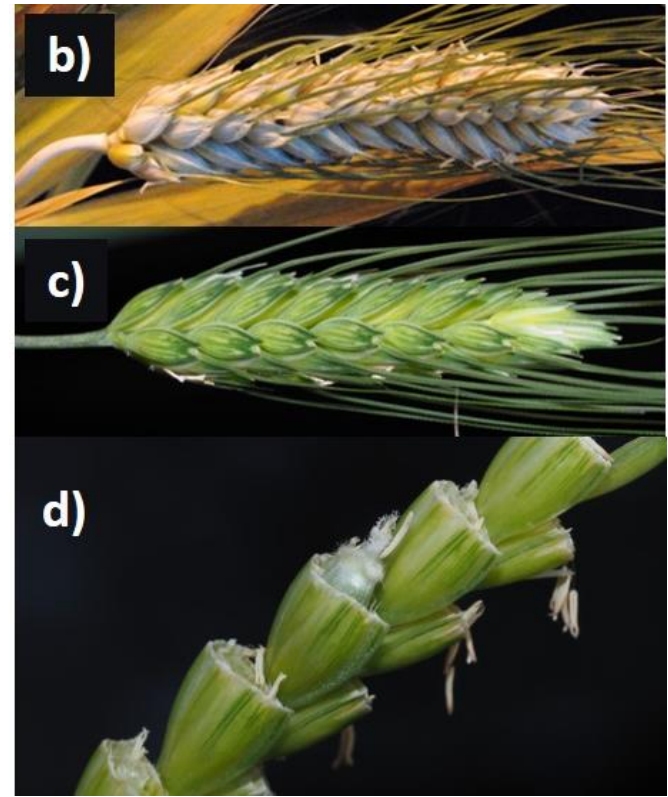


Figure 18: Crossing strategy for Introgression of *T. monococcum* into Hexaploid Wheat. Please note that all germplasms shown on the left are used as the females, to be pollinated with the fertile anthers of the male germplasms on the right (as indicated by the gender signs).

Table 5: No of F₁ stigmas pollinated with Paragon and 'F₁ complex' grains obtained

	MDR031	MDR049	MDR308
Kronos	960 stigmas	120 stigmas	120 stigmas
Grains (% of stigmas)	7 (0.73%)	0	1 (0.83%)
Hoh501	1920 stigmas	400 stigmas	none
Grains (% of stigmas)	9 (0.47%)	0	n/a



a) Comparison of heights of triploid F_1 plant (left) and Hoh501 parent **b)** selfed Hoh501 grain-filled ear **c)** 'selfed' Hoh501 x MDR031 F_1 sterile ear (empty) **d)** ripening 'F₁ complex' grain. Note extruded anthers in c) and d) which never contained any pollen.

Germination of F₁ Complex Grains

#	cross	germinated	vernalisation	growth	ears	BC ₁ F ₁ grains	selfed grains
24	[Kronos x MDR031] _x [Paragon]	YES	YES		7	9	
25		NO					
27			YES				
34							
40	[Kronos x MDR308] _x [Paragon]						
46-1	[Kronos x MDR031] _x [Paragon]		YES				
46-2			YES				
47			YES		5	5 (1; 4)	1
66	[Hoh501 x MDR031] _x [Paragon]		YES				
77							
82			YES				
84							
86-1			YES		6	Too early	
86-2			YES		5	Too early	
94			YES		4	Too early	
108							
112		1 of 3 seeds	YES				

F₁ hybrid:
Kronos x MDR031



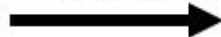
x Paragon (♂)



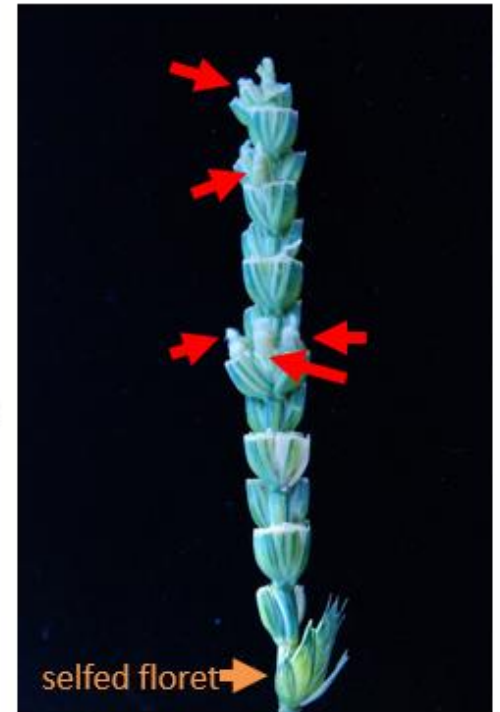
F₁C#24



x Paragon (♂)



F₁C#24BC₁



ear shape & phenotype	Very close to Kronos	Very close to Paragon
anthers	Extruding, NO pollen	Extruding, pollen , partially self-fertile