Wheat
Genetic
Improvement
Network

John Innes Centre

Anchorage and Drought Update

Clare Lister and Simon Griffiths

08/10/2019

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Drought Trial Update

2016				2017				2018				2019					
Spring Rain, little Summer Drought				Spring Drought, some Summer Drought				Spring Rain, severe Summer Drought				Spring Rain, some Summer Drought					
Opposite Drier				Riverside				Football				Opposite Drier				Rain-fed	Irrigated
Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Garcia	Paragon

Booting		Booting		Booting		Booting		X		X		X		X		
178	2B	178	2B	178	2B											
						193	2B									
203	2D	203	2D	203	2D	204	2D									
402	4D															RhtD1
				494	5B											
687	7A	688	7A	687	7A	687	7A									

Booting		Booting		Booting		Booting		X		X		X		X		
	2B		2B		2B											
							2B									
	2D		2D		2D		2D									
	4D															RhtD1
					5B											
	7A		7A		7A		7A									

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Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Garcia	Paragon

DTEM		DTEM		DTEM		DTEM		DTEM		DTEM		DTEM		DTEM		Partial data	
178	2B	178	2B	178	2B							178	2B			NIL production	
						193	2B										
204	2D			203	2D	204	2D							203	2D		
								237	3A	240	3A						
				494	5B												
687	7A	688	7A	687	7A	690	7A	687	7A	687	7A	690	7A	690	7A		
								731	7B	731	7B						

DTEM		DTEM		DTEM		DTEM		DTEM		DTEM		DTEM		DTEM		Partial data	
	2B		2B		2B								2B			NIL production	
							2B										
	2D				2D		2D								2D		
									3A		3A						
					5B												
	7A		7A		7A		7A		7A		7A		7A		7A		
									7B		7B						

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Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Garcia	Paragon

HT		HT		HT		HT		HT		HT		HT		HT			
16	1A	16	1A	16	1A	16	1A					16	1A	16	1A	NIL production	
286	3B	292	3B			292	3B										
401	4D	401	4D	401	4D	401	4D	401	4D	401	4D	401	4D	401	4D	RhtD1	
										455	5A						
										494	5B			494	5B		
						700	7A			698	7A			700	7A		

HT		HT		HT		HT		HT		HT		HT		HT			
	1A		1A		1A		1A						1A		1A	NIL production	
	3B		3B				3B										
	4D		4D		4D		4D		4D		4D		4D		4D	RhtD1	
											5A						
											5B				5B		
							7A				7A				7A		

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Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Garcia	Paragon

YLD		YLD		YLD		YLD		YLD		YLD		YLD		YLD		
						18	1A									
												82	1D			
				173	2B	173	2B	176	2B	176	2B					NIL production
												220	2D			
										571	6A					
743	7B															

YLD		YLD		YLD		YLD		YLD		YLD		YLD		YLD		No YLD QTLs
							1A									
													1D			
					2B		2B		2B		2B					NIL production
													2D			
											6A					
	7B															

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Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Garcia	Paragon

SW		SW		SW		SW		SW		SW		SW		SW		
						20	1A									
				145	2B											PpdB1?
177	2B					173	2B	177	2B							NIL production
232	3A															
403	4D	401	4D	402	4D	401	4D	400	4D	401	4D	401	4D	401	4D	RhtD1
		441	5A													
450	5A	455	5A													
				781	7D											

SW		SW		SW		SW		SW		SW		SW		SW		
							1A									
					2B											PpdB1?
	2B						2B		2B							NIL production
	3A															
	4D		4D		4D		4D		4D		4D		4D		4D	RhtD1
			5A													
	5A		5A													
					7D											

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TGWT		TGWT		TGWT		TGWT		TGWT		TGWT		TGWT		TGWT		
15	1A	13	1A	15	1A			13	1A	13	1A	13	1A			NIL production
294	3B															
		401	4D	401	4D	401	4D			401	4D			401	4D	RhtD1
												415	5A			
437	5A	437	5A													
				456	5A	456	5A									
		482	5B	483	5B	483	5B	482	5B	482	5B	482	5B	476	5B	
				690	7A	690	7A	690	7A							

TGWT		TGWT		TGWT		TGWT		TGWT		TGWT		TGWT		TGWT		
	1A		1A		1A				1A		1A		1A			NIL production
	3B															
			4D		4D		4D				4D				4D	RhtD1
													5A			
	5A		5A													
					5A		5A									
			5B		5B		5B		5B		5B		5B		5B	NIL production?
					7A		7A		7A							NIL production?

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Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Marker	Chr	Garcia	Paragon

Grains / m2		Grains / m2		Grains / m2		Grains / m2		Grains / m2		Grains / m2		Grains / m2		Grains / m2		No Grains / m2 QTLs
												14	1A			
						142	2B	141	2B	141	2B			142	2B	PpdB1?
222	2D															
				401	4D	401	4D	401	4D	401	4D	400	4D	401	4D	RhtD1
						418	5A									
				493	5B							493	5B			
691	7A			690	7A			690	7A			690	7A			

Grains / m2		Grains / m2		Grains / m2		Grains / m2		Grains / m2		Grains / m2		Grains / m2		Grains / m2		No Grains / m2 QTLs
													1A			
							2B		2B		2B				2B	PpdB1?
	2D															
					4D		4D		4D		4D		4D		4D	RhtD1
							5A									
					5B								5B			
	7A				7A				7A				7A			

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Anchorage

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- **WP3 - YIELD STABILITY TRAITS:**

- **WP3.1 Spring Drought tolerance**
- **Obj4** Screen parents of existing mapping populations and genetic stocks for drought tolerance

- **WP3.2 Lodging Resistance and Anchorage**
- **Obj1** Screen parents of existing mapping populations and genetic stocks for lodging resistance
- **Obj2** Screen parents of existing mapping populations and genetic stocks for anchorage testing
- **Obj3** Develop procedure for testing anchorage strength

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DALP

- Combined all of these Objectives to create **DALP**

DROUGHT, ANCHORAGE and LODGING PANEL

- CIMMYT lines
- SATYN panel
- Elite varieties
- Roth Res and JIC lines
- DFW Breeders TK H17 and H18
- EMS semi-dwarves
- Paragon x Garcia RILs
- Watkins – few
- Parents of populations

DALP

- **DALP**
- Grown as 1 m plots 2018-19
- Seed bulking
- DTEM / HT / (YLD)
- Lodging – close to harvest

- Development of anchorage method + / - irrigation – bit too close to harvest…

Anchorage

- Develop method for testing anchorage
- No ideas on measurement from WGIN community!
- Roland Ennos and Mitch Crook advice – future collaboration with MC?
- Needs to be easy and quick to do for large-scale screening
- Timing – after anthesis (and later?)
- + / - irrigation
- Many readings required – variation in plots

Helped by Charlie Philp, Scott Sheldrick, David Norris, James Nott!

Anchorage

- Spring balance
- Pull force gauges (range 25N – 200N)
- Plastic twine and knots X
- Natural twine and knots X
- Natural twine and clips ✓
- + / - Irrigation

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Anchorage

- Spring balance

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- Pull force gauges (range 25N – 200N)

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- Need very secure attachment to plants!

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Anchorage

- Irrigation helps!

2019 Trial

- ~ 230 lines + / - irrigation
- Spaced plants (~ 5.5. cm apart)
- 6m plots so able to irrigate with boom
- Usual phenotyping (DTEM / HT / YLD)
- Lodging scores
- Will sacrifice specified segment of plots to anchorage tests

Nested Association Mapping Population Selection for Lodging Trial

David Norris
Clare Lister
Simon Griffiths

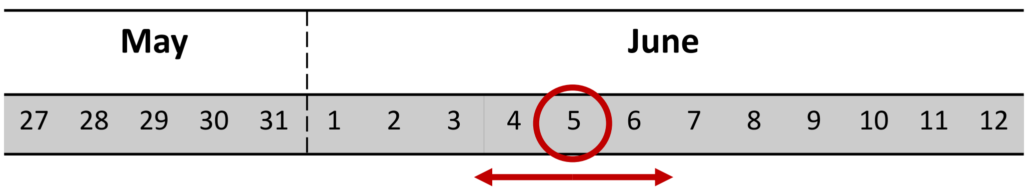
Assessing lodging traits from Paragon NAM background

- Nested association mapping (NAM) population
- WGIN and DFW have 80+ populations of crosses between Paragon and founder parents (including the Watkins collection)
- With a minimum of 96 RILs per founder parent
- Trial objective: gene discovery - finding marker associated traits for lodging with high accuracy
- Efficient scan of the genetic variation

How did we select RILs?

- From each population of 96 RILs, we selected a sub-set of 11
- Aim to select RILs with phenotypes more similar to Paragon through 3-step process:

1) DTEM



2) Height

3) Yield

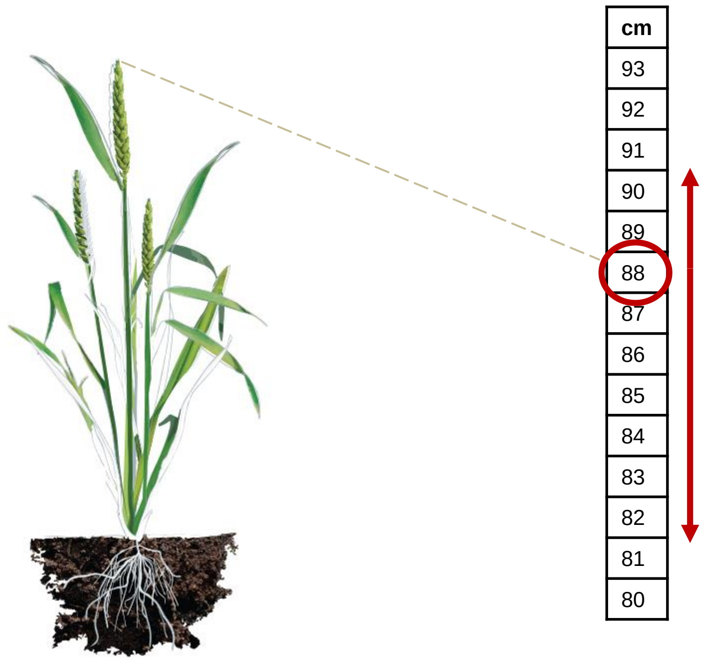
From each population of 96 RILs, we selected 11

- Aim to select RILs with phenotypes more similar to Paragon through 3-step process:

1) DTEM

2) **Height**

3) Yield



From each population of 96 RILs, we selected 11

- Aim to select RILs with phenotypes more similar to Paragon through 3-step process:

1) DTEM

2) Height

Yield (kg)									
0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1	1.2

3) **Yield**

Example of selecting 11 RILs one population - (Paragon x Watkins 496)

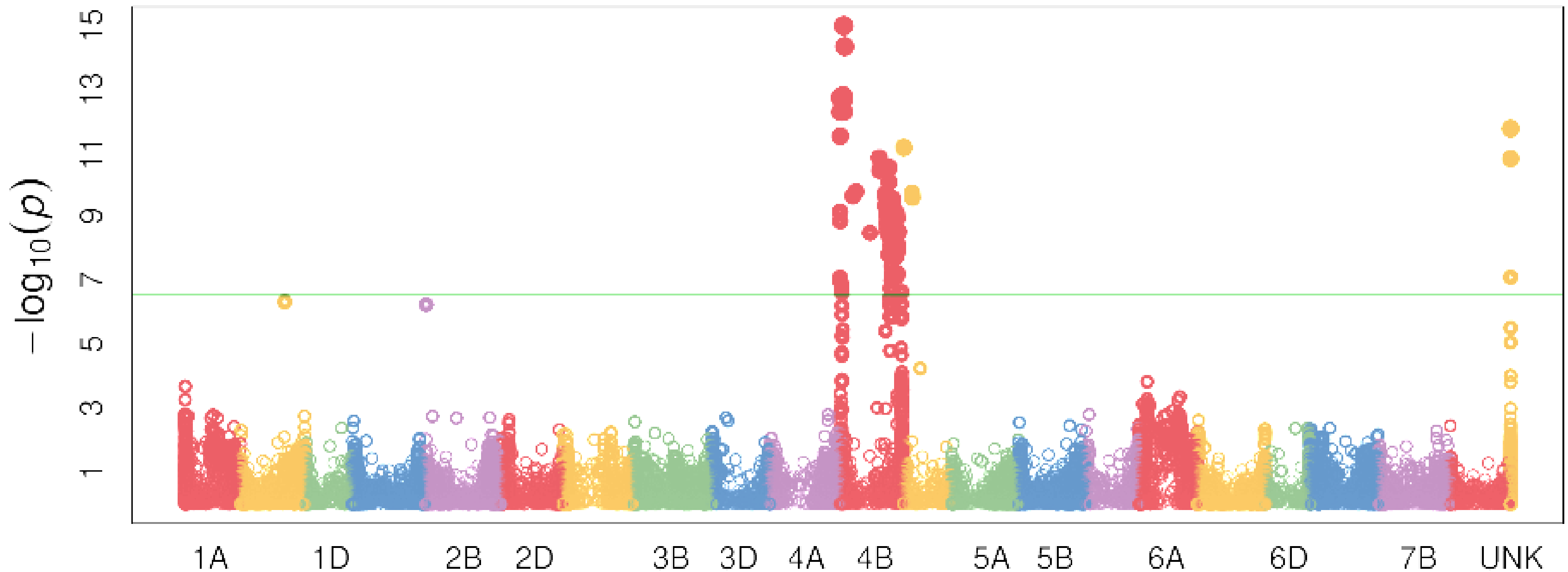
I.D	Difference in height to Paragon (cm)	Difference in DTEM to Paragon (days)	Difference in Yield to Paragon (kg)	Selected?
PxW496-44	+5	-4	-0.24	Y
PxW496-54	+4	-4	-0.3	Y
PxW496-65	+8	-4	0.02	Y
PxW496-68	+6	-4	-0.3	
PxW496-16	+6	-3	-0.06	Y
PxW496-18	+8	-3	-0.12	Y
PxW496-20	+10	-3	-0.06	
PxW496-22	0	-3	-0.08	Y
PxW496-24	+2	-3	-0.22	Y
PxW496-39	+15	-3	-0.39	
PxW496-66	+4	-3	-0.12	Y
PxW496-90	-2	-3	-0.16	Y
PxW496-49	+12	-2	-0.8	
PxW496-79	-8	-2	-0.7	
PxW496-42	+9	-1	-0.16	Y
PxW496-53	+1	-1	-0.32	
PxW496-41	+1	0	-0.08	Y

Trial design and measurements

- Germination tests on all lines, selection adjustment
- After selection process, settled on 466 RILs for the trial
- Drilled shortly in 1m plots, randomized design
- Phenotyping:
  - Standard measurements: height, DTEM, yield
  - Lodging
  - Stiffness

And what our results could look like….

Plant Height



Summary

- Selected 466 RILs from 52 founder parent crosses with Paragon

- Selection criteria prioritised RILs with similar DTEM and height phenotypes to Paragon

- Trial aims to find marker associated traits for lodging

WGIN management

Tuesday 8th Oct

JIC

Paragon library Rht-1 focus

What I thought I knew about *Rht-1*

Fig. 1. Effects of different *Rht* combinations on plant height. Photograph shows the tall control, *Rht-B1b*, *Rht-D1b*, *Rht-B1b* + *Rht-D1b*, *Rht-B1c* and *Rht-B1c* + *Rht-D1b* lines of Maris Huntsman, from left to right.

Journal of Agricultural Science, Cambridge (1997), **128**, 11–25. © 1997 *Cambridge University Press* 11

Optimizing wheat grain yield: effects of *Rht* (gibberellin-insensitive) dwarfing genes

J. E. FLINTHAM[1], A. BÖRNER[2], A. J. WORLAND[1] AND M. D. GALE10[1]

[1] *John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK*

[2] *Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstraße 3, D-06466 Gatersleben, Germany*

(*Revised MS received 28 March 1996*)

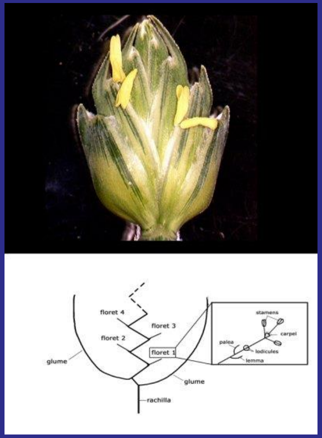
Classic descriptions of Rht-1 yield enhancing effect

Table 3. *Genotype means for grain yield (g m^{-2}) in different field trials*

Variety	Line	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Genotype mean
Maris Huntsman	Tall control	218	415	731	752	836	915	645
	Rht-B1b	244	437	779	772	918	948	683
	Rht-D1b	237	455	776	812	875	929	681
	Rht-B1b + Rht-D1b	201	417	711	712	843	948	639
	Rht-B1c	186	251	642	632	824	861	566
	Rht-B1c + Rht-D1b	116	164	465	508	706	528	415
Maris Widgeon	Tall control	159	404	528	600	683	732	518
	Rht-B1b	206	429	603	721	781	829	595
	Rht-D1b	212	411	538	723	786	852	587
	Rht-B1b + Rht-D1b	178	368	494	650	774	749	536
	Rht-B1c	185	337	542	574	726	762	521
	Rht-B1c + Rht-D1b	140	257	419	465	585	577	407
Bersée	Tall control	206	367	574	606	743	771	544
	Rht-B1b	223	452	626	754	894	907	643
	Rht-D1b	214	404	598	705	859	891	612
	Rht-B1b + Rht-D1b	210	344	601	778	890	830	609
	Rht-B1c	203	396	546	664	862	830	584
	Rht-B1c + Rht-D1b	157	269	513	569	753	727	498
April Bearded	Tall control	156	205	354	409	513	431	345
	Rht-B1b	239	228	460	460	600	572	426
	Rht-D1b	218	241	416	550	618	617	443
	Rht-B1b + Rht-D1b	223	417	501	633	751	727	542
	Rht-B1c	242	436	532	627	696	691	537
	Rht-B1c + Rht-D1b	196	288	430	507	640	634	449
Trial mean		199	350	557	633	757	761	543

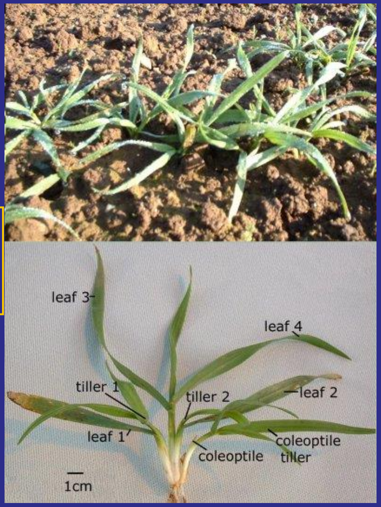
Similar for Maringa background- Miralles and Slafer 1995

Components of grain yield in wheat

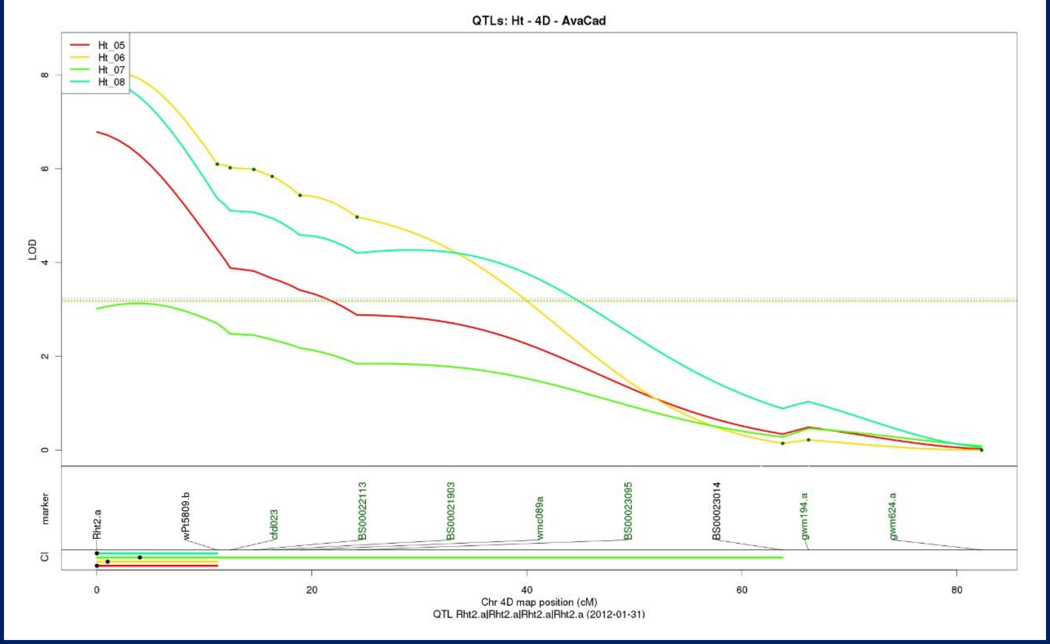


Grain yield

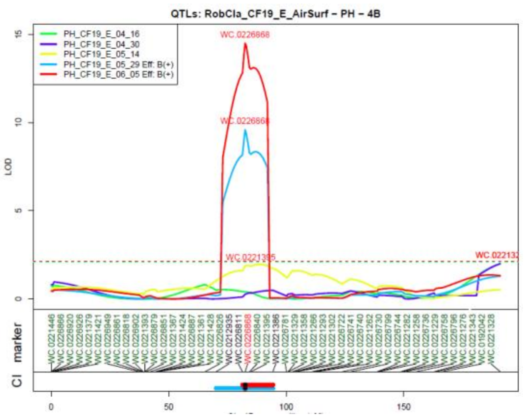
- Grains m^2
  - Grains spike^{-1}
    - Grains spikelet^{-1}
    - Spikelets spike^{-1}
  - Spikes m^2
    - Spikes Plant^{-1}
    - Plants m^2
- Grain size

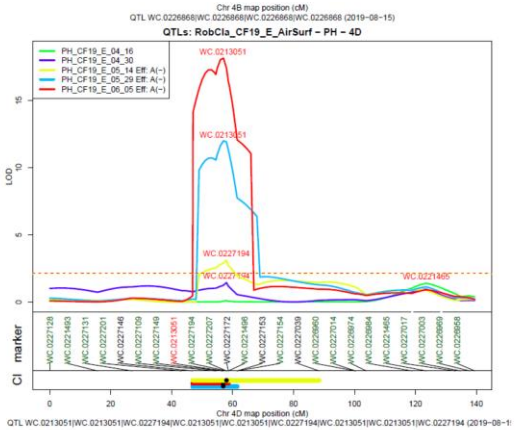


Avalon Cadenza showed us that you don't always see the whole phenotypic complex



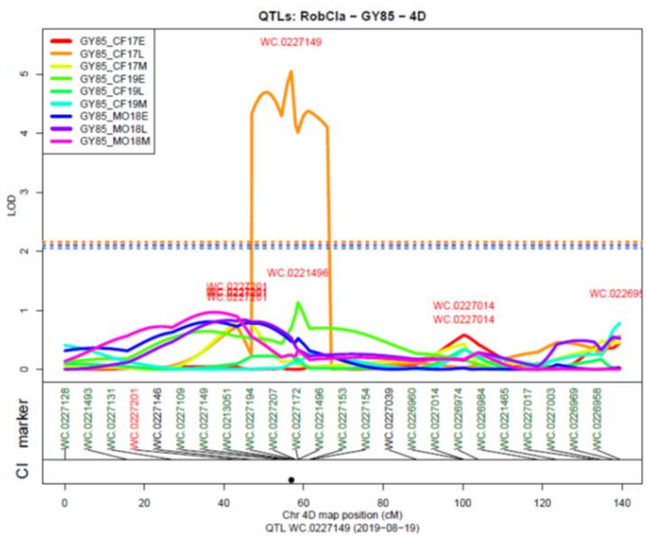
- First UK reference population established by WGIN
- Segregates for *Rht-D1* (*Rht2*)
- Rht-1 traits:
  - Height YES
  - Thousand grain weight YES
  - Yield NO





This is true in many segregating populations in this case Robigus x Claire

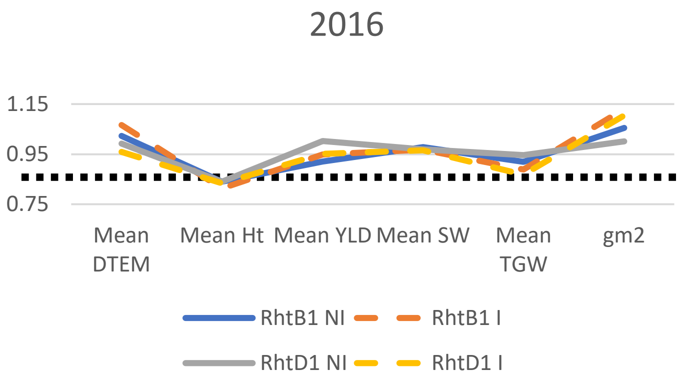
Robigus x Claire – height, low tgw etc but no yield

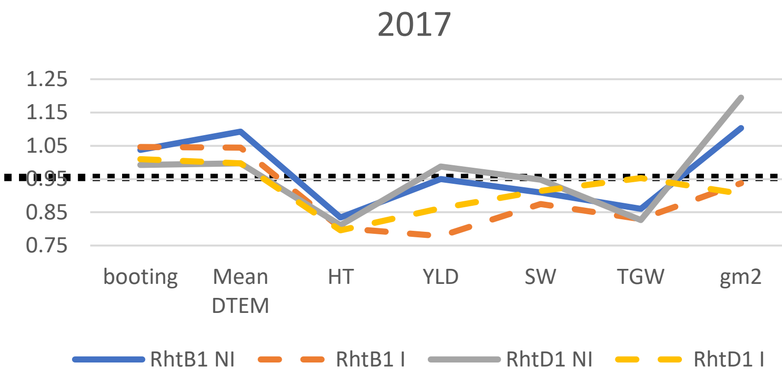


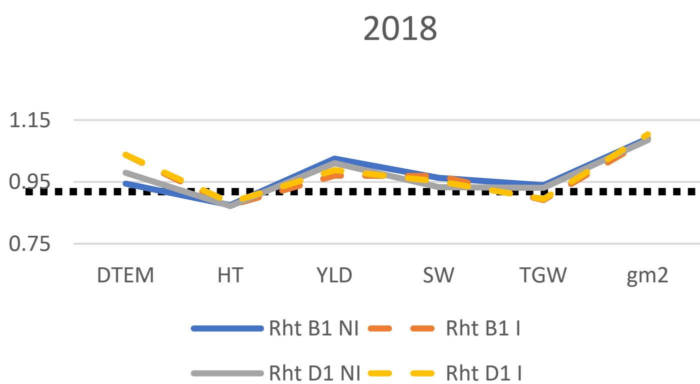
- There is a yield QTL for Rht-D1 in late drilled material
- The Robigus allele is increasing!
- Why?

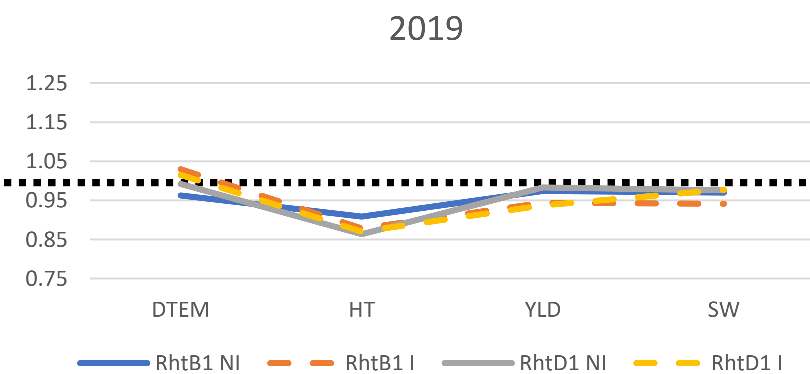
chr	LOD	%var	mean	add eff	trait	env	population	increasing Allele
4D	4.9	11.3	5.246	-0.489	GY	CF17_L	RobCla	A

The WGIN NIL libraries are when way to see what might control expression of Rht-1 yield effects



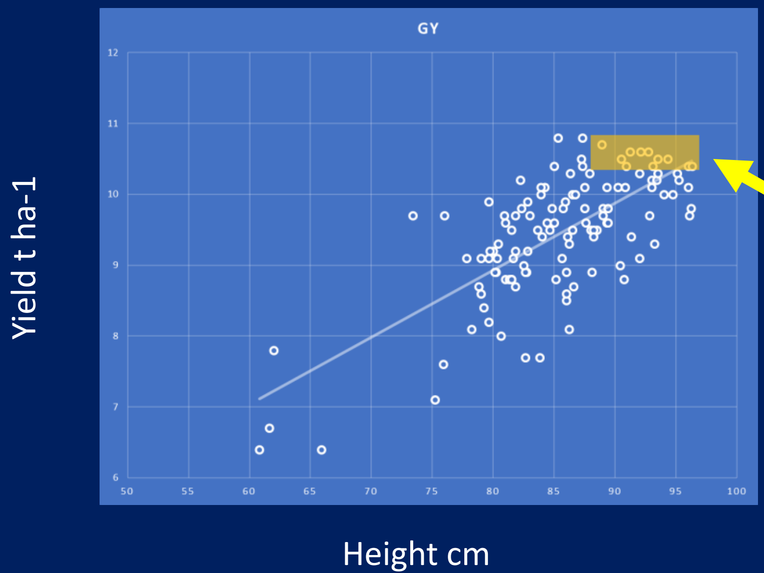






This is also the year that we got a significant yield increasing QTL in Paragon x Garcia

Maybe UK breeding does need some new dwarfing genes?

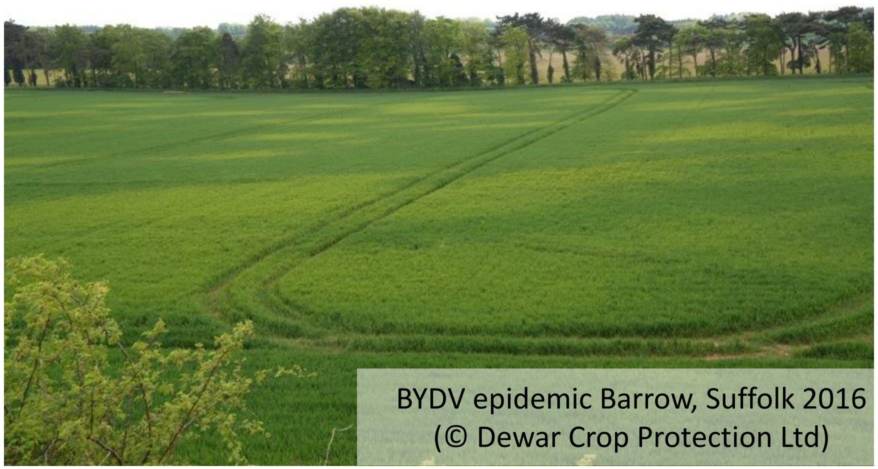


- Paragon EMS mutants chosen from 7000 for:
    - Good agronomic type
    - Reduced height
    - This was WGIN work
- Taken on in DFW for replicated yield trials (3x 6m2) at high yield potential
- The Paragon window height x yield window
- Some of the lines have significant height decreases compared to Paragon and (non significant) yield increases.
- In DFW we are repeating the trial and in WGIN introducing these lines into DALP

Aphids and BYDV

Gia Aradottir

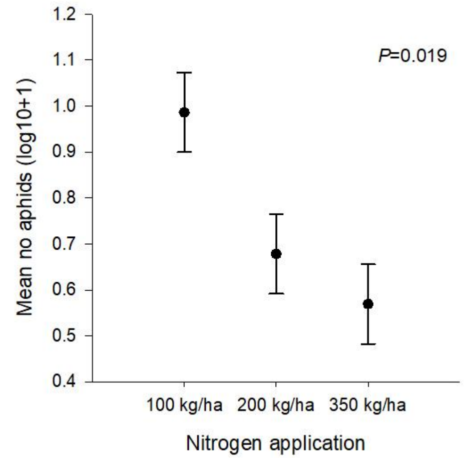
ROTHAMSTED RESEARCH

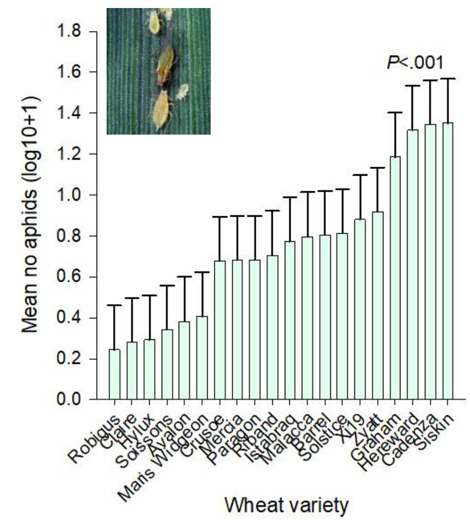


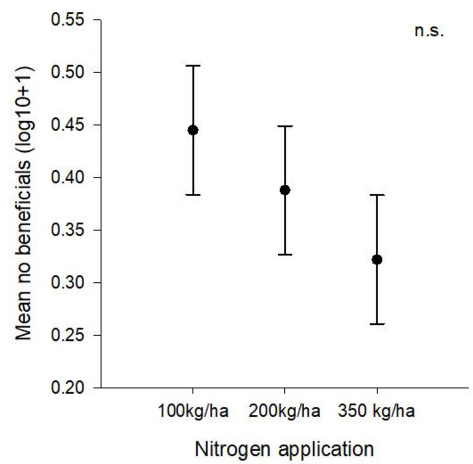
BYDV epidemic Barrow, Suffolk 2016
(© Dewar Crop Protection Ltd)

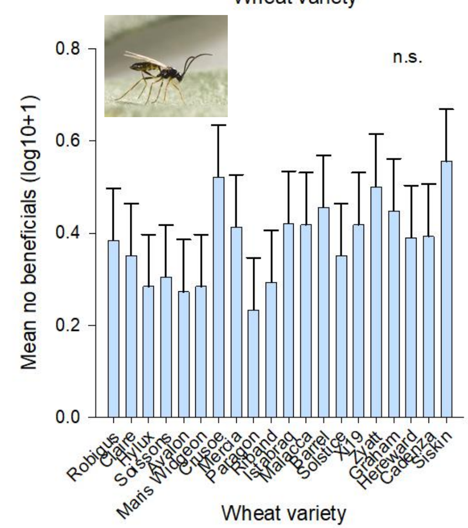
WGIN – Diversity trial 2019

- No BYDV symptoms recorded in field
- Difference in aphid numbers between varieties
- Also difference between with N levels (contrary to literature)
- Beneficials counted but no difference btw N level or variety



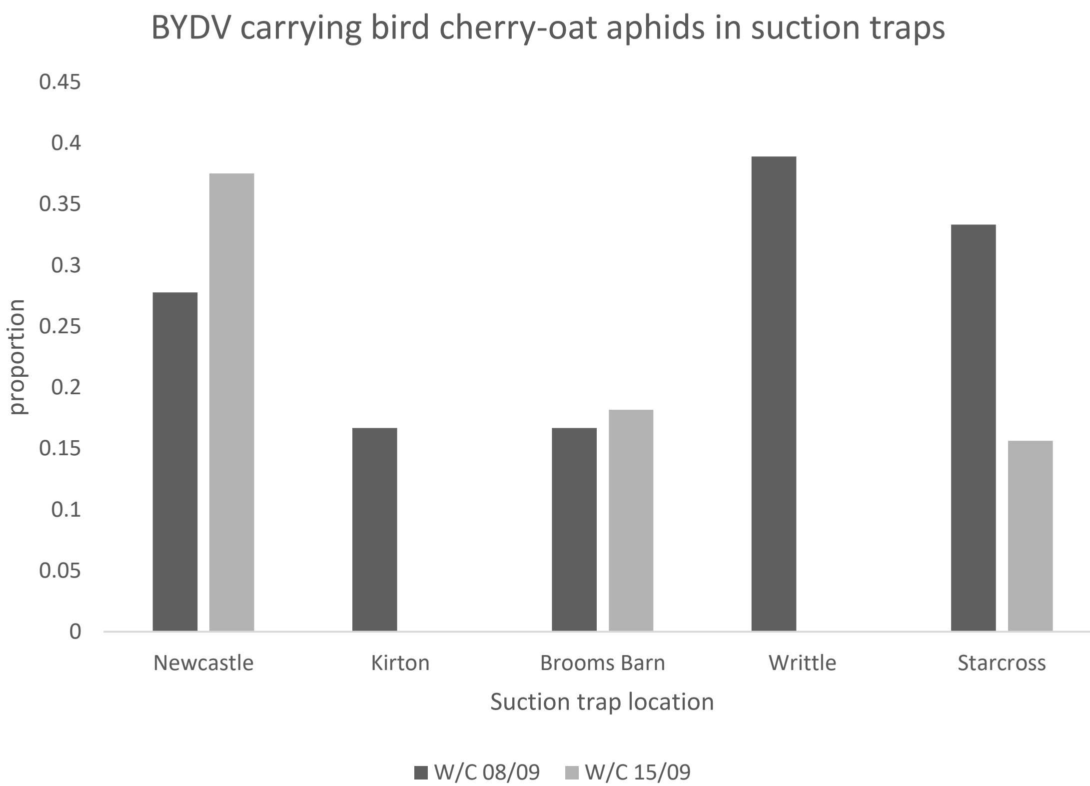




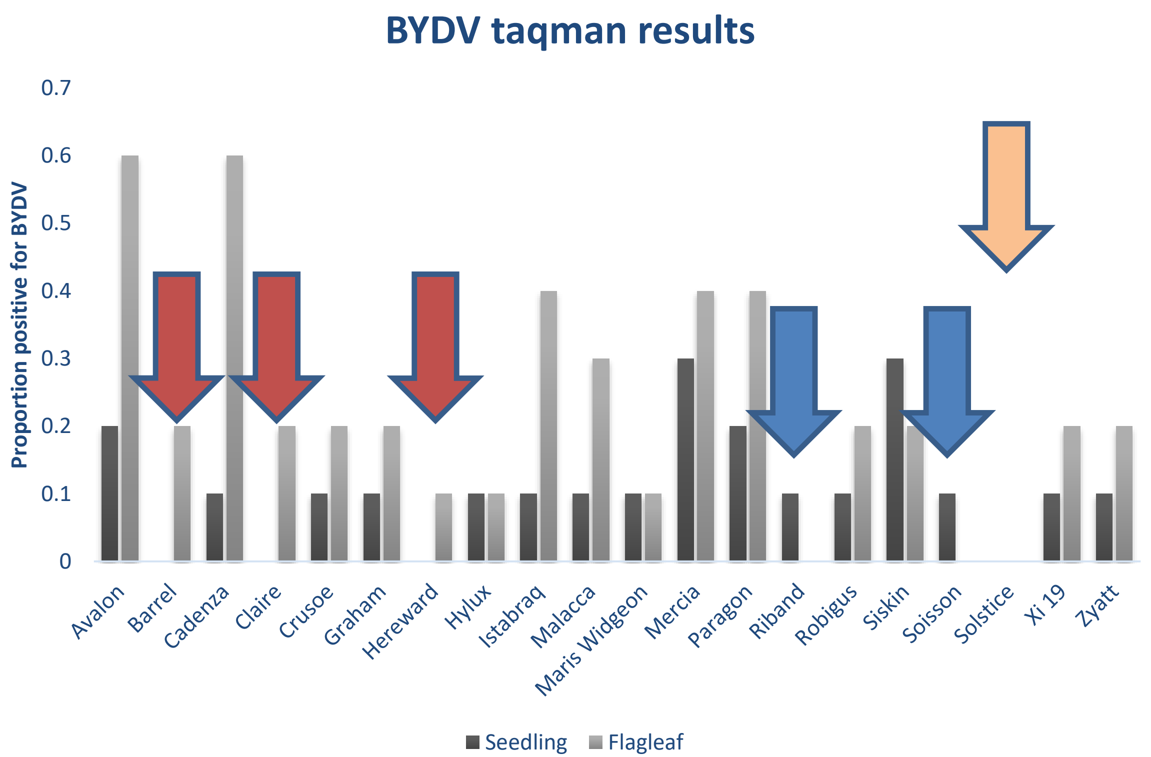


BYDV prevalence in UK cereal aphids

- The Rothamsted Insect Survey and AHDB now report prevalence of BYDV carrying aphids in ‘Aphid news’
- www.ahdb.org.uk/aphid-news to subscribe
- So far Autumn 2019, avg 24% of bird cherry-oat aphids carry the virus
- Only one English grain aphid recorded which carried BYDV



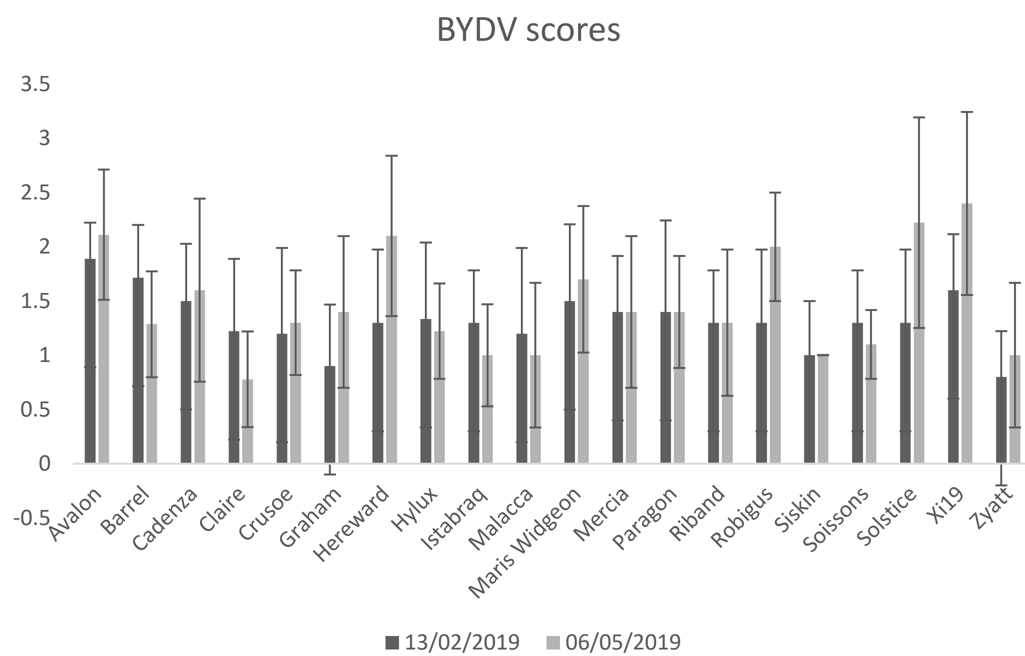
Virus testing (taqman)



- 5 BYDV carrying aphids put onto one week old plant and left to feed for one week before insecticide treatment
- First samples taken one week after insecticide treatment
- Second samples take at flagleaf stage
- Lower infection rates than expected
- Solstice had no infection

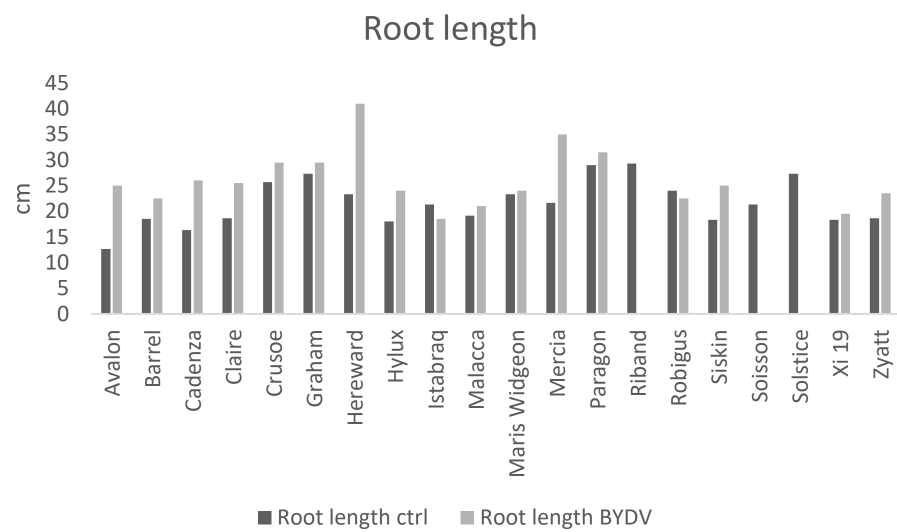
Visual scoring - Diversity lines

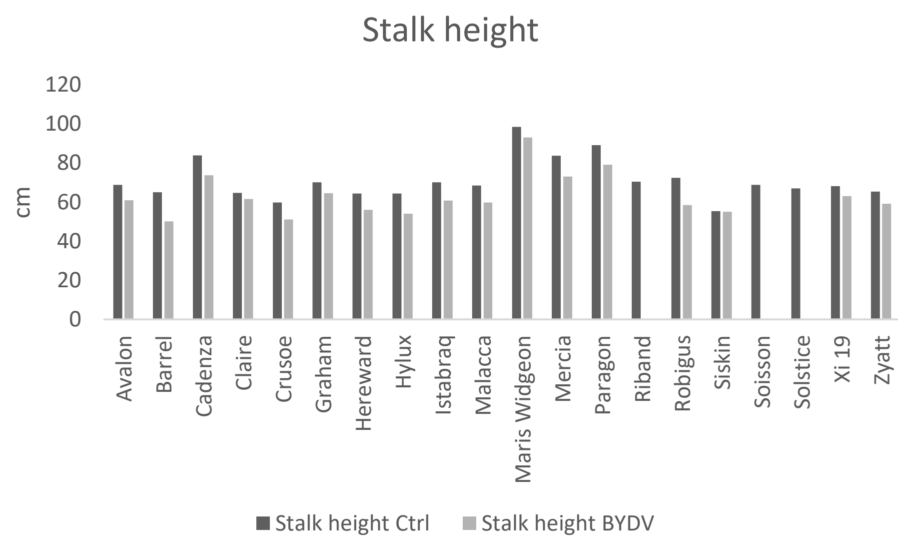
- Initial scoring presented at last meeting does not match with taqman assays
- Some plants had yellowing circles on centre of leaf blade, others yellowing at tips
- Samples taken from distal end of leaf may miss infection?



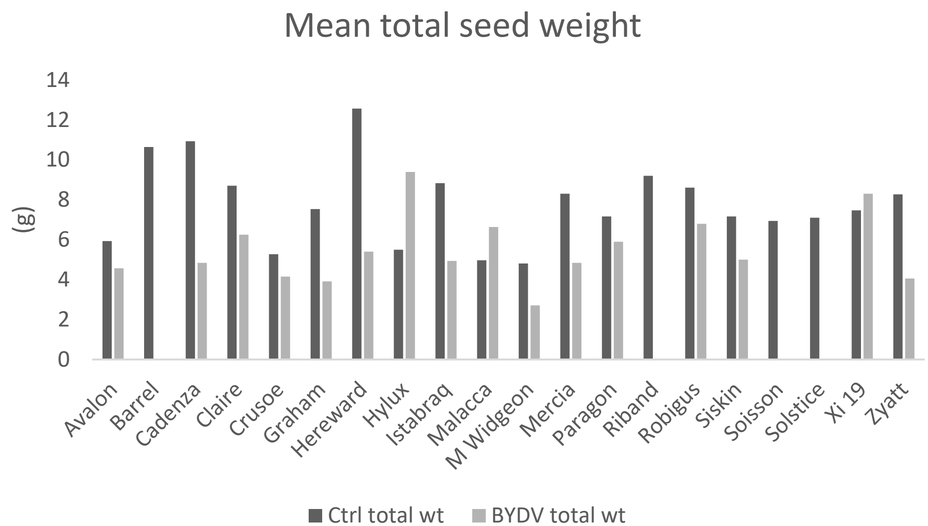
Plant measurements

- Root length and stem length measured
- Control plants 3 reps
- BYDV infected at flagleaf vary in no from 1 - 6

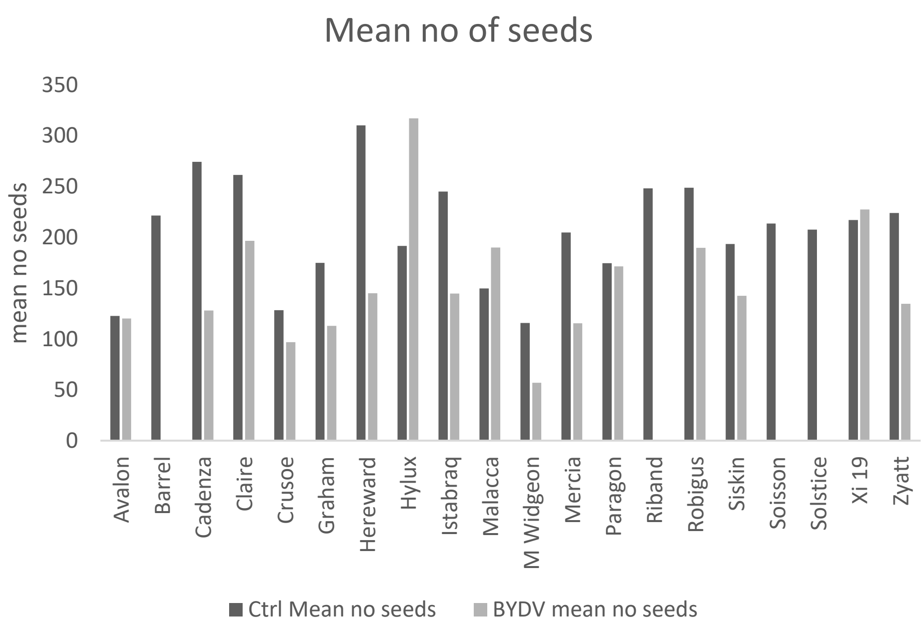


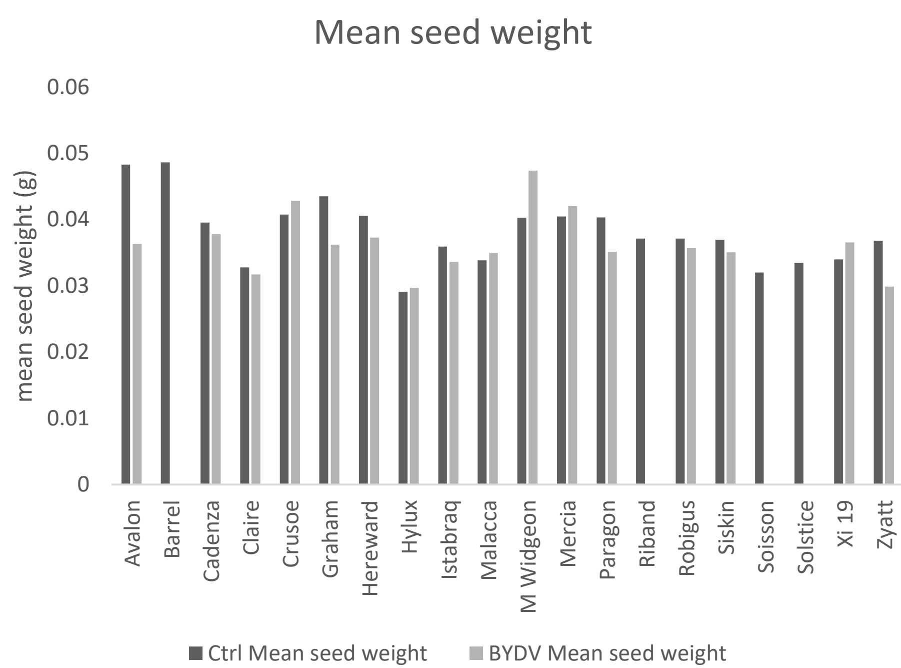


Seed no & weight



Variety	BYDV N=	Ctrl N=
Avalon	6	3
Barrel	0	2
Cadenza	6	3
Claire	2	3
Crusoe	2	3
Graham	2	3
Hereward	1	3
Hylux	1	3
Istabraq	3	3
Malacca	3	3
Maris Widg	1	3
Mercia	3	3
Paragon	3	3
Riband	0	2
Robigus	2	3
Siskin	2	3
Soisson	0	3
Solstice	0	3
Xi 19	2	3
Zyatt	2	3





Further batches infected with BYDV

BYDV carrying aphids have been put onto 50 more lines

These are in the glasshouse and vernaliser

Leaf samples have been taken and stored in freezer for taqman analysis

In glasshouse

Watkins collection
WATDE0007
WATDE0032
WATDE0034
WATDE0042
WATDE0044
WATDE0045
WATDE0079
WATDE0103
WATDE0110
WATDE0126
WATDE0127
WATDE0139
WATDE0141
WATDE0145
WATDE0160
WATDE0468
WATDE0729
Paragon
Hereward
Solstice

In vernaliser

NIAB synthetics

WS0001 SHW012	WS0025 SHW082
WS0003 SHW018	WS0026 SHW083
WS0004 SHW027	WS0027 SHW084
WS0005 SHW028	WS0028 SHW085
WS0006 SHW029	WS0029 SHW086
WS0007 SHW030	WS0030 SHW087
WS0008 SHW031	WS0033 SHW090
WS0010 SHW035	WS0034 SHW091
WS0011 SHW036	WS0036 SHW093
WS0012 SHW042	WS0037 SHW094
WS0014 SHW051	WS0038 SHW095
WS0015 SHW054	WS0039 SHW096
WS0017 SHW071	WS0040 SHW099
WS0018 SHW072	WS0042 SHW137
WS0019 SHW073	WS0043 SHW138
WS0021 SHW076	Hereward
WS0022 SHW077	Paragon
WS0024 SHW080	Solstice

Future work

- Variation in aphid infestation on Diversity lines – and depending on N application (contrary to literature, where higher N = more aphids)
- Interesting results from Diversity infection trial
  - some varieties did not pick up BYDV (Solstice)
  - Some had high rates (Cadenza, Avalon)
  - Some plants had the virus at seedling stage but not at flagleaf stage!
- Visual scoring for BYDV not reliable and needs studying
- Plants in glasshouse and vernaliser need scoring and sampling
- Seed of ~30 wild relatives (parents of alien introgressions from Nottingham) at Rres to be tested

Wgin Diversity Trial Update

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Reduced pesticide treatment

Date	Operation	Active ingredients	Std Farm Practice	Reduced rate
09/10/2018	Drilled			
27/11/2018	Insecticide		Yes	No
28/02/2019	N + S		Yes	Yes
03/05/2019	Nitogen		Yes	Yes
03/05/2019	T1	Epoxiconaxole, isopyrazam, chlorothalonil	Yes	No
15/05/2019	Nitrogen		Yes	Yes
23/05/2019	T2	Tebuconazole, epoxiconazole, fluxapyroxad, pyraclostrobin	Yes	No
26/06/2019	T3	Prothioconazole, tebuconazole & spiroxamine	Half rate	Quarter rate

- Disease scored 16/05/19 for Septoria, yellow rust, necrosis
- Handheld images collected 17/05/19 @ 90 and 45 degrees
- 06/06/19 flag leaf only, yellow & brown rusts, Septoria, necrosis
- Low altitude images collected 26-28/06/19 under different lighting conditions @ 90 and 45 degrees
- 26/06/19 assess diseased/necrotic tissue to coincide with low altitude aerial images (top leaf, 2nd and 3rd leaf assessed separately)
- Hyperspectral reflectance measured weekly through growing season
- Drone imagery collected weekly Jan-Jul
- Also collected low altitude images from a fusarium experiment (11/07/19)

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Example images

Images collected at nadir and 45 degrees 17/05/19

Low altitude aerial images collected 26-28/06/19

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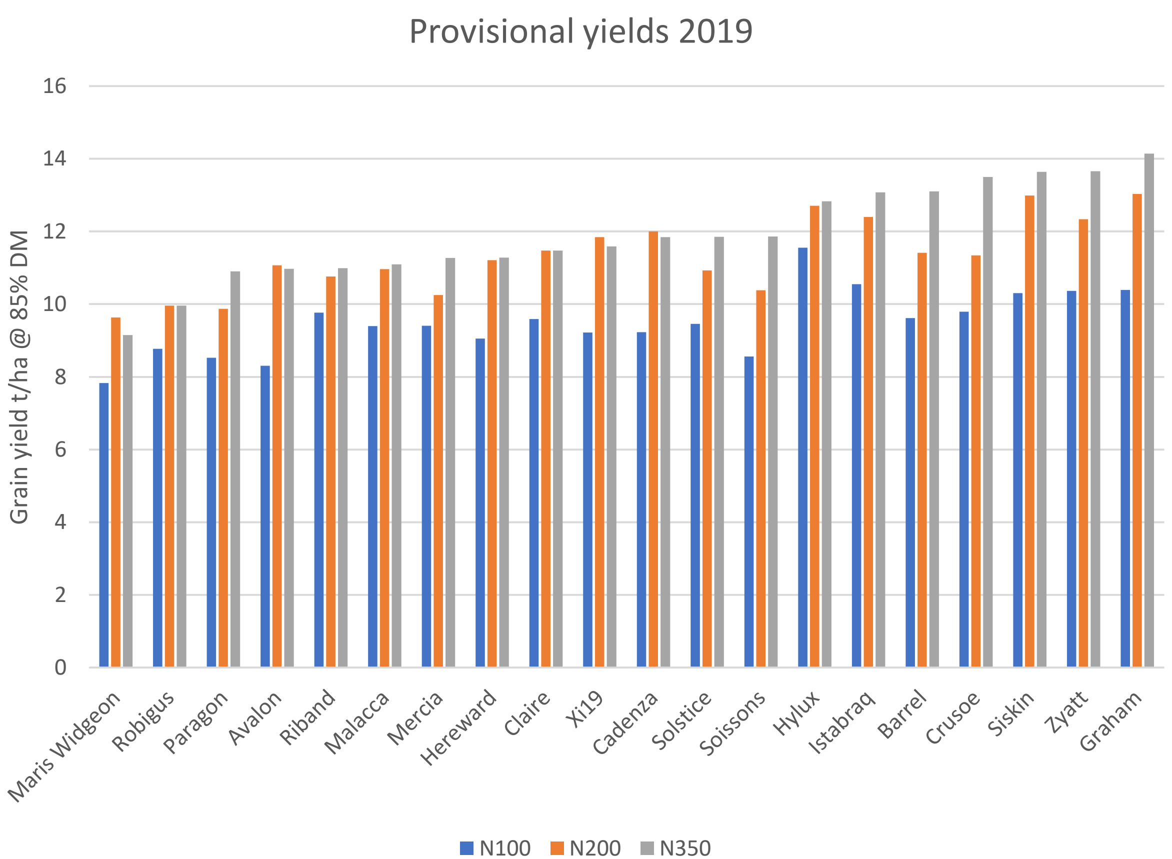
Treated yield 2019



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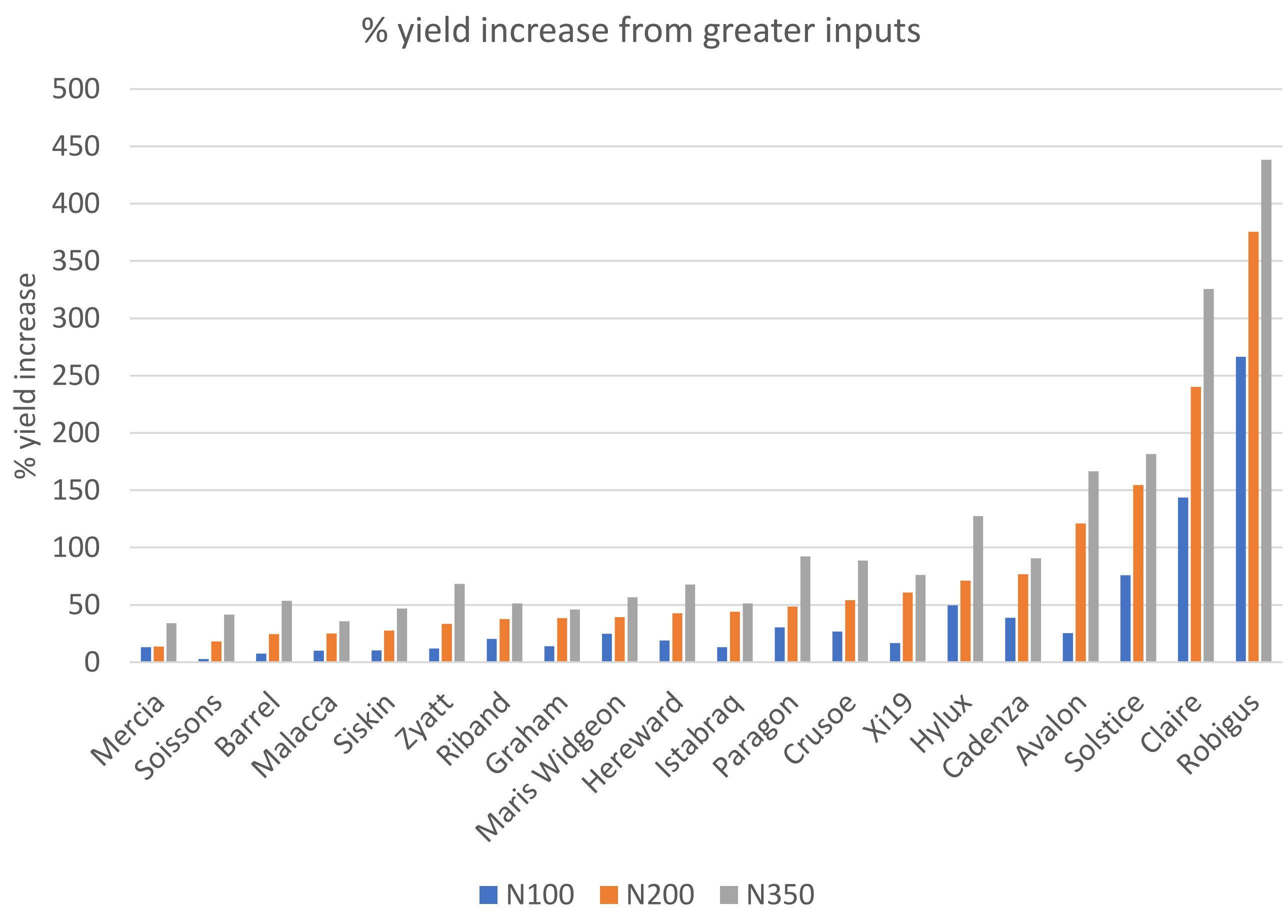
Yield increase from increased inputs



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2020 Diversity trial

Avalon	Maris Widgeon
Barrel	Mercia
Cadenza	Paragon
Claire	Riband
Crusoe	Robigus
Graham	Siskin
Hereward	Soissons
Theodore	Solstice
Istabraq	Xi19
Malacca	Zyatt

3 levels of N

Standard farm practice and reduced pesticide inputs

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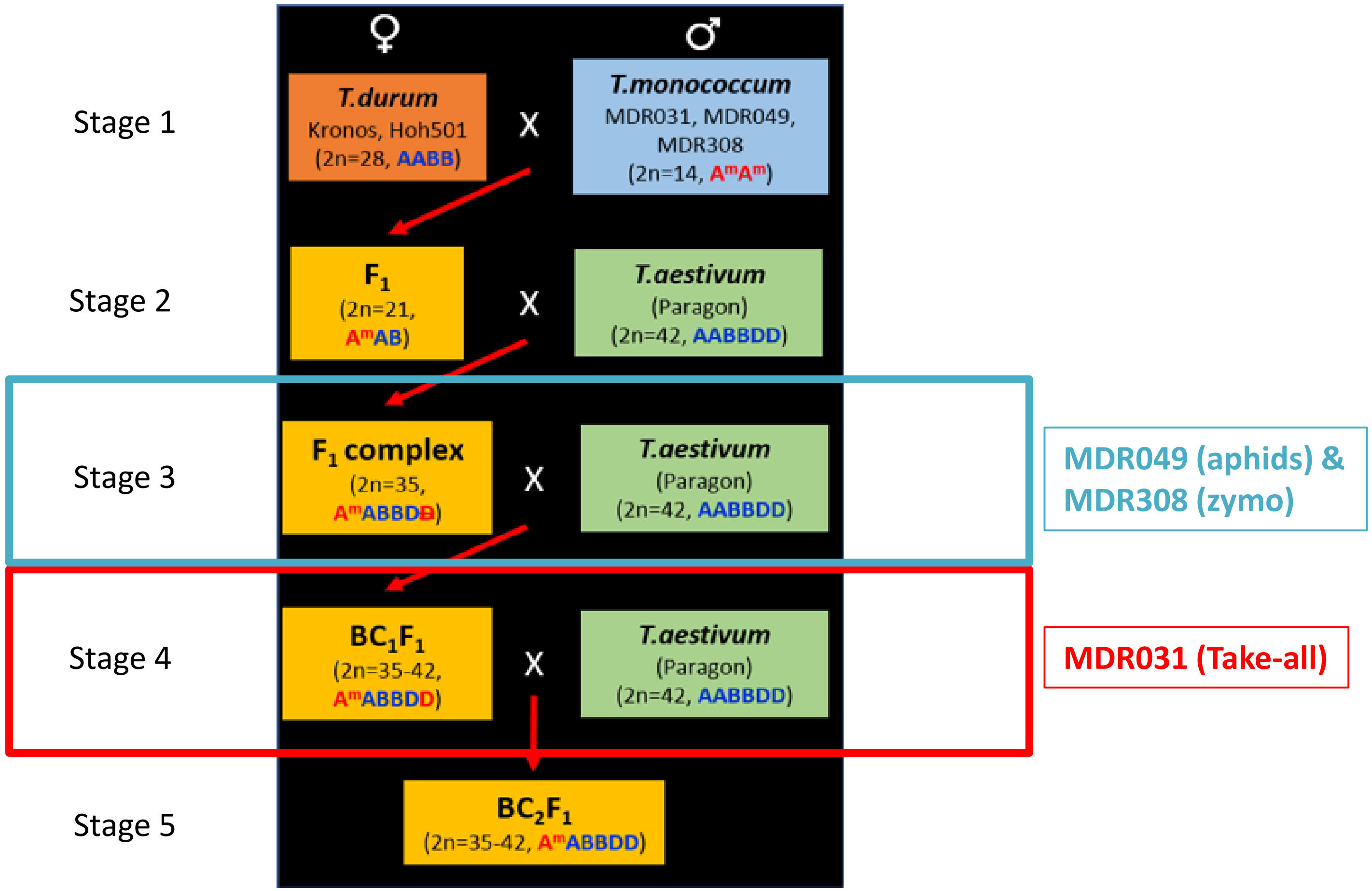
Tm Introgression

Mike Hammond-Kosack

WGIN MM October 8th 2019

@JIC


Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
♀
♂
T.durum
Kronos, Hoh501
(2n=28, AABB)
X
T.monococcum
MDR031, MDR049, MDR308
(2n=14, AmAm)
F1
(2n=21, AmAB)
X
T.aestivum
(Paragon)
(2n=42, AABBDD)
F1 complex
(2n=35, AmABBD~~B~~)
X
T.aestivum
(Paragon)
(2n=42, AABBDD)
BC1F1
(2n=35-42, AmABBDD)
X
T.aestivum
(Paragon)
(2n=42, AABBDD)
BC2F1
(2n=35-42, AmABBDD)
MDR049 (aphids) & MDR308 (zymo)
MDR031 (Take-all)

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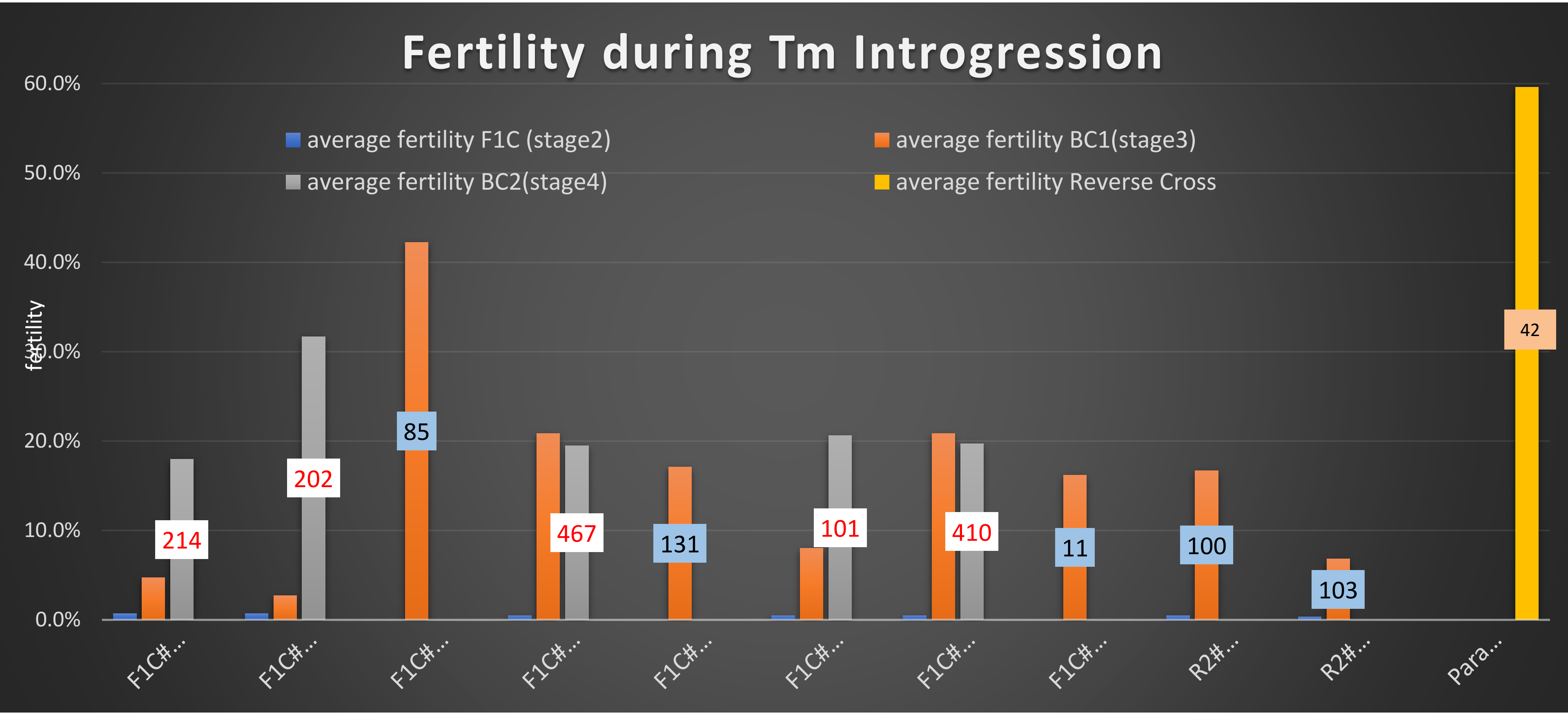

BC_1 grains generated:

1. round 2 for MDR049 & MDR308 – **F_1C#MDR*** (♀) x Paragon (♂)**,
2. selfed F_1C#MDR031 plants: **F_1C#***S (♀) x Paragon (♂)**,

grains generated from reverse cross: **Paragon (♀) x BC_1-F_1C# (♂)**

BC_2 grains generated: **BC_1-F_1C# (♀) x Paragon (♂)**

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BC_1 grains generated:
1. round 2 for MDR049 & MDR308 – **F_1C#MDR*** (♀) x Paragon (♂)**
2. selfed F_1C#MDR031 plants: **F_1C#***S (♀) x Paragon (♂)**,

1
- **100** grains from [Hoh501 (♀) x **MDR049** (♂)]
- **103** grains from [Hoh501 (♀) x **MDR308** (♂)]

2
- **85** grains from [**Kronos (♀)** x MDR031 (♂)]
- **142** grains from [**Hoh501 (♀)** x MDR031 (♂)]

BC_2 grains generated:
BC_1-F_1C#MDR031 (♀) x Paragon (♂)

- **416** grains from [**Kronos (♀)** x MDR031 (♂)]
- **978** grains from [**Hoh501 (♀)** x MDR031 (♂)]

grains generated from reverse cross: **Paragon (♀) x BC_1-X147-1 (♂)**

- **42** grains from [**Kronos (♀)** x MDR031 (♂)]

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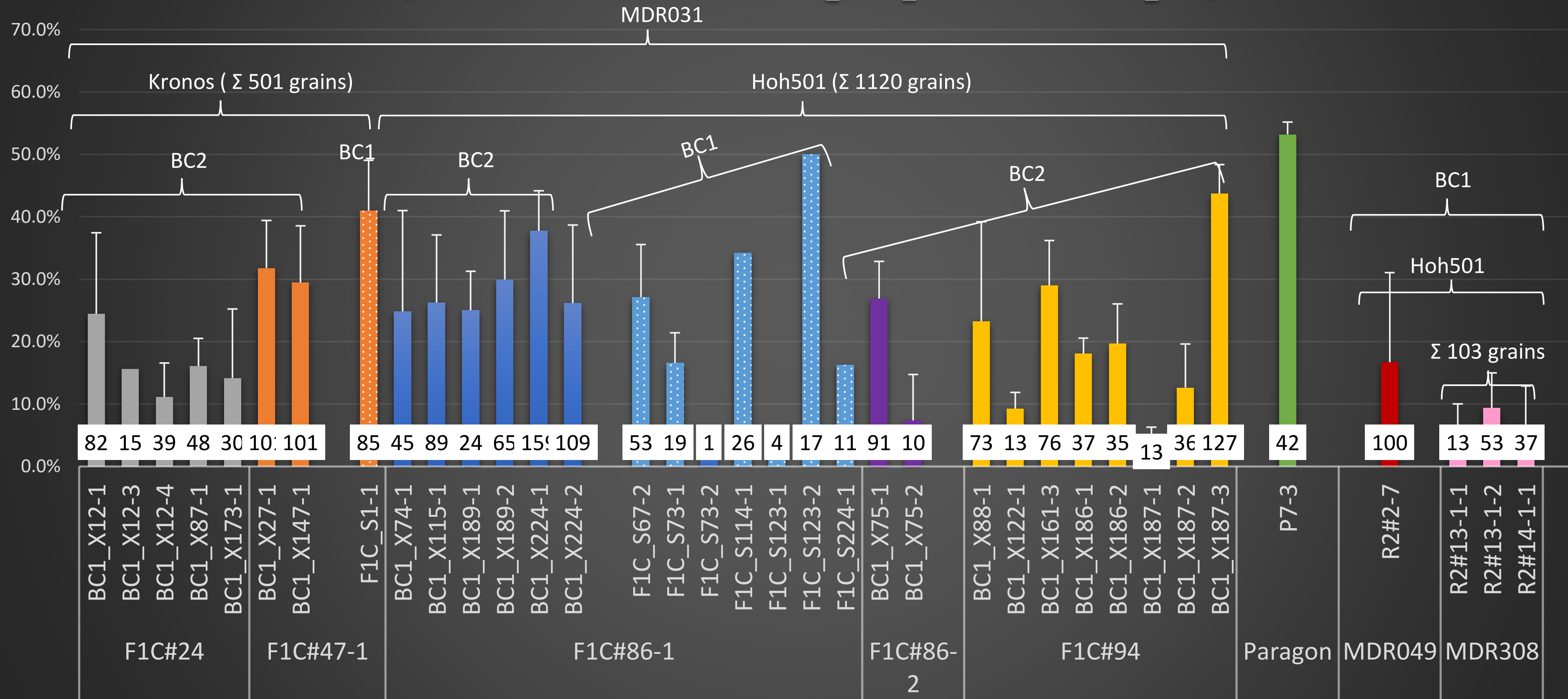
fertility of individual BC_1, F_1C-S and F_1C plants



black numbers = total no. of grains obtained for BC_2 crosses on individual BC_1 plants (BC_1 for R2 plants)

BC_1_X12-3 = plant grown from grain no.3 from back cross no.12 with F_1Complex plant F_1C#24 (♀) x Paragon (♂)
F_1C_S73-1 = plant grown from grain no.1 of **selfed bottom floret (cross73)** of F_1Complex plant F_1C#86-1,
R2#2-7 = **round 2** of plant grown from grain no.7 of F_1Hybrid plant 2 (♀) x Paragon (♂)[=F_1Complex]

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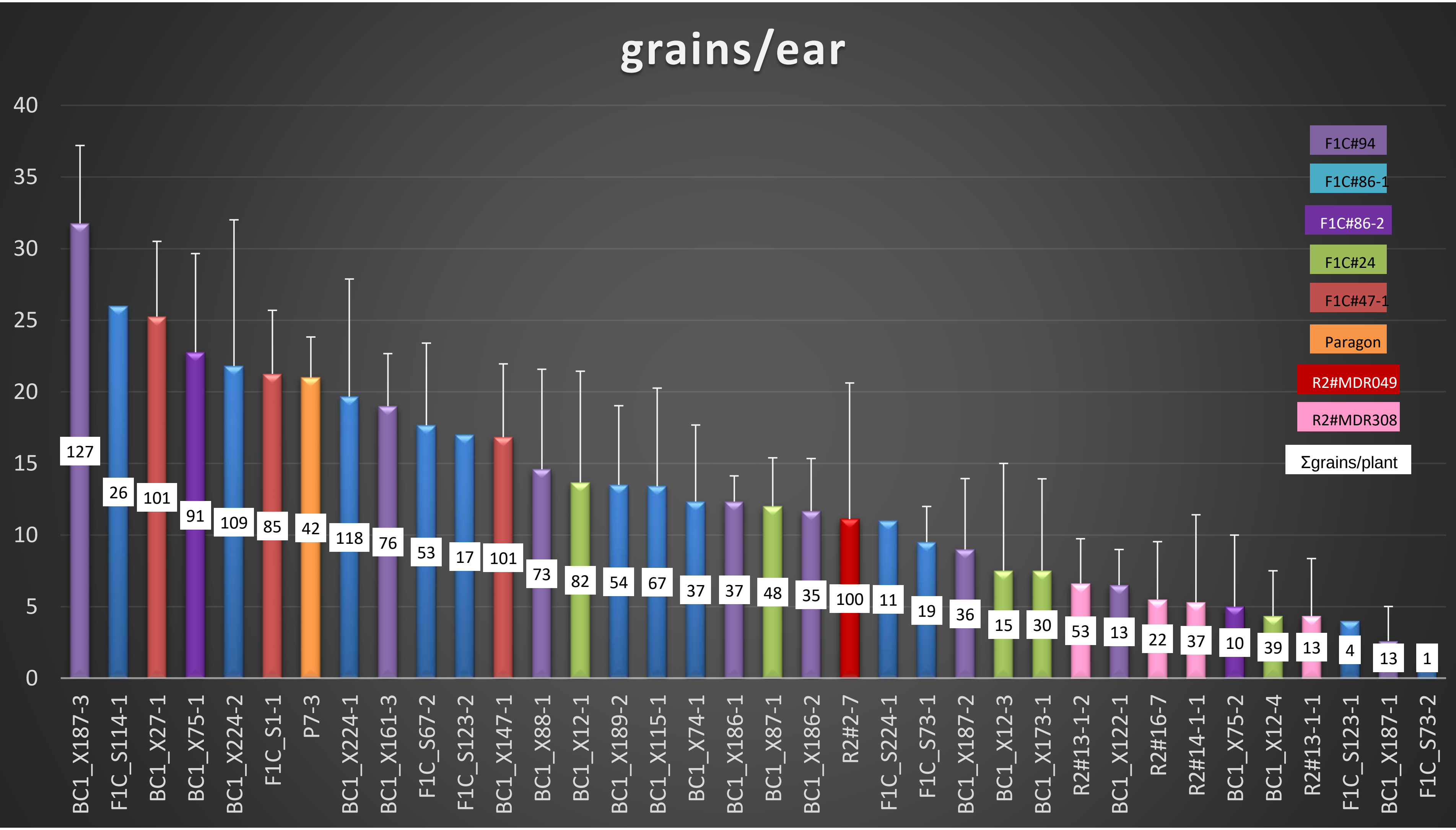

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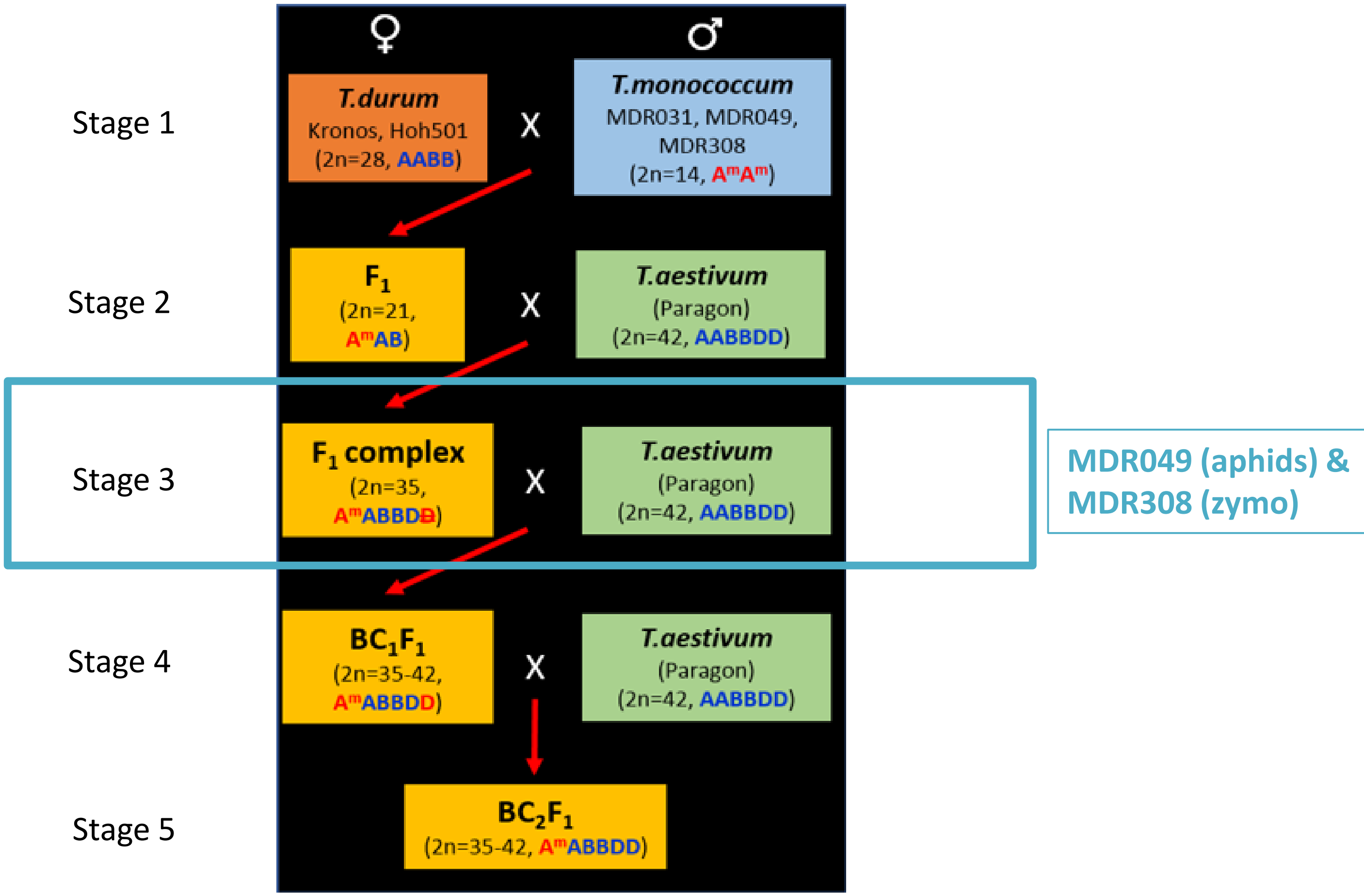

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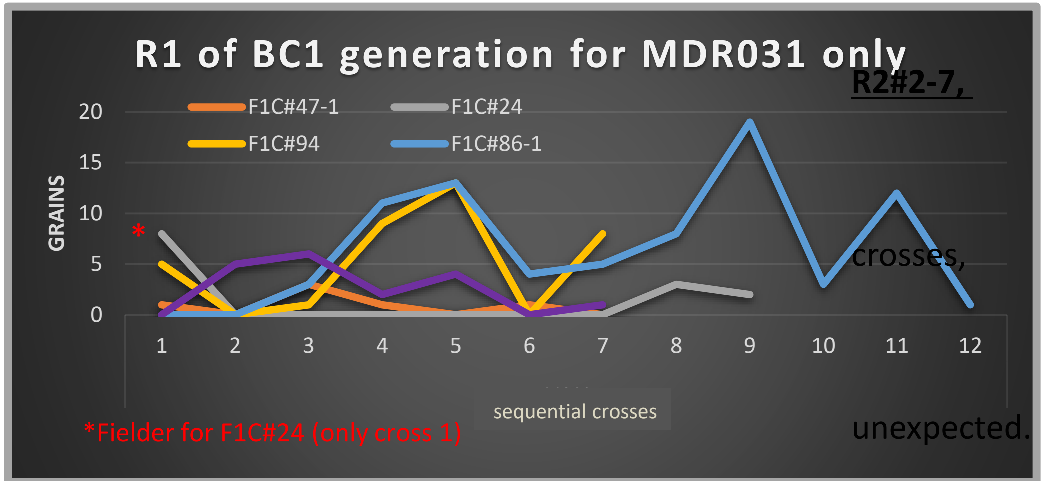

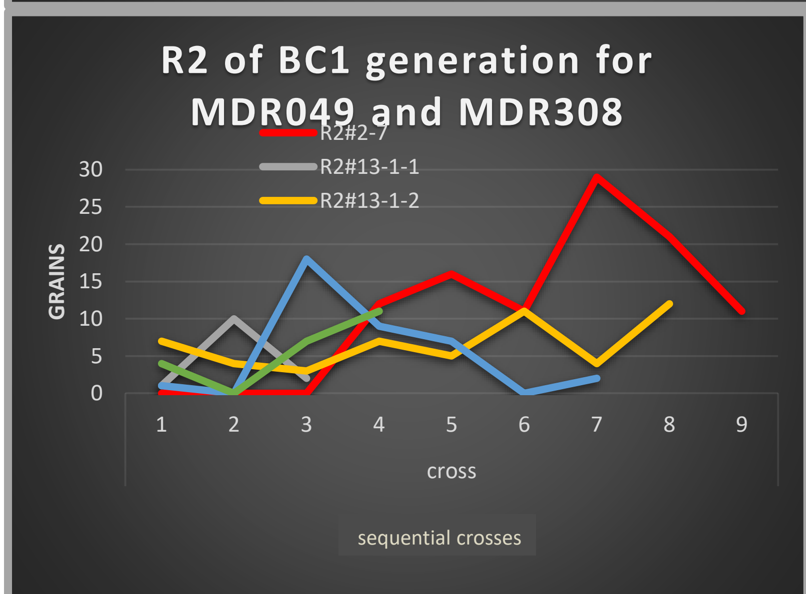

<u>unusual 'behaviour' of three R2 plants - R2#2-7, R2#13-1-1 & R2#16-7</u>

nb: all R2 plants are F1C plants

1) R2#2-7 (MDR049) - **NO** BC1 grains for first 3 crosses, then suddenly very high numbers

2) all selfed ears (bagged, but otherwise untouched) have **tons of grains**, which is unexpected.

3) **R2#16-7** has a Paragon appearance on selfed ears, but the 4 crosses carried out only generated 4, 0, 7 and 11 grains, ie well below expected numbers if it were some hexaploid variety.

4) The number of BC1 grains generated from these 3 R2 plants is **higher** than for MDR031 in the previous experiment, especially for MDR049 (R2#2-7) where cross 7 generated 29 grains.

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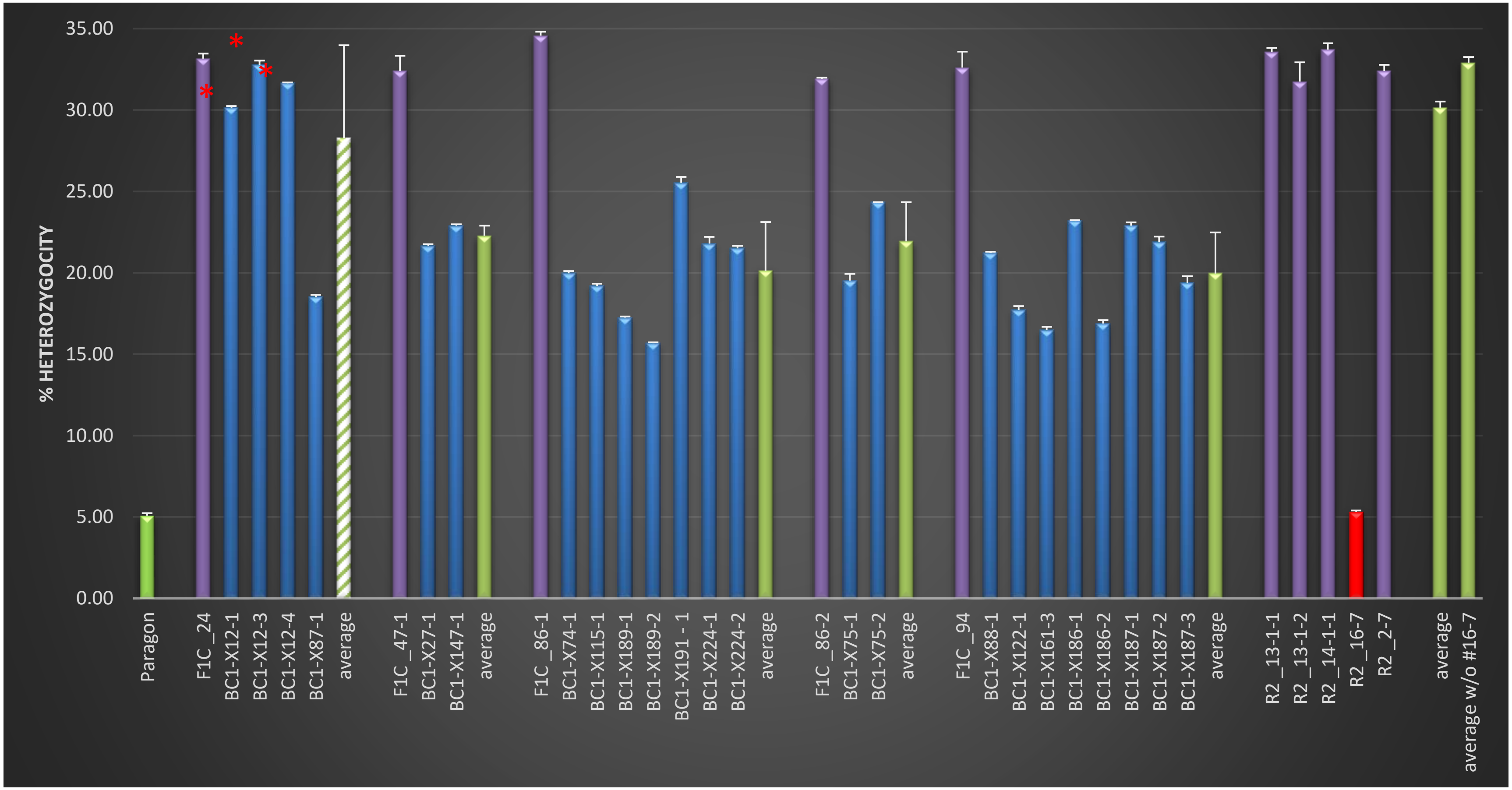
Heterozygosity of F1C and BC1 plants (from Axiom 35k Breeders' Array Genotyping)



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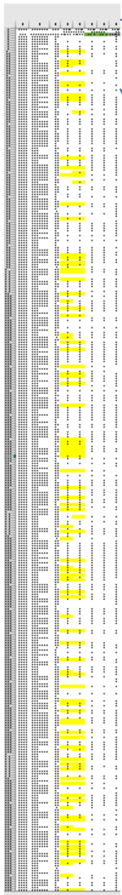
R2_16-7 is NOT Paragon



1	probeset_id	Conversion Type	Call Rate	K21_R2 _16-7.C	L22_R2 _16-7_r	A21_P20-4.CEL	B22_P20-4_repl
2				most likely Paragon		Paragon	
3	proid	Conversion Type	Call Rate	R2 _16-7	R2 _16-7(r)	P20-4	P20-4(r)
4	AX-94381147	PolyHighResolution	98.9619	0	0	0	0
5	AX-94381170	PolyHighResolution	99.654	0	0	0	0
6	AX-94381257	NoMinorHom	99.654	2	2	2	2
7	AX-94381264	NoMinorHom	97.5779	0	0	0	0
8	AX-94381285	Other	87.8893	2	2	2	2
9	AX-94381295	NoMinorHom	96.5398	2	2	2	2
10	AX-94381338	Other	96.1938	1	1	0	0
11	AX-94381384	PolyHighResolution	94.8097	2	2	2	2
12	AX-94381419	Other	56.7474	-1	0	-1	0
13	AX-94381436	Other	99.654	0	0	0	0
14	AX-94381448	OTV	97.9239	2	2	0	0
15	AX-94381449	PolyHighResolution	96.8858	2	2	0	0
16	AX-94381454	PolyHighResolution	96.5398	-1	0	0	0
17	AX-94381470	NoMinorHom	97.9239	0	0	0	0
18	AX-94381476	MonoHighResolution	100	2	2	2	2
19	AX-94381488	Other	90.6574	0	0	1	1
20	AX-94381509	NoMinorHom	97.5779	0	0	0	0
21	AX-94381520	PolyHighResolution	100	0	0	0	0
22	AX-94381525	NoMinorHom	95.8478	0	0	0	0
23	AX-94381554	Other	93.7716	0	0	2	2
24	AX-94381612	PolyHighResolution	97.2318	2	2	0	0
25	AX-94381626	NoMinorHom	98.2699	0	0	0	0
26	AX-94381628	PolyHighResolution	98.6159	2	2	2	2
27	AX-94381637	PolyHighResolution	92.3875	0	0	0	0
28	AX-94381641	Other	98.9619	0	0	1	1
29	AX-94381646	PolyHighResolution	94.8097	1	1	2	2
30	AX-94381659	Other	92.0415	2	2	1	1
31	AX-94381712	PolyHighResolution	99.308	2	2	2	2
32	AX-94381736	OTV	96.5398	2	2	2	2
33	AX-94381754	PolyHighResolution	92.0415	0	0	0	0

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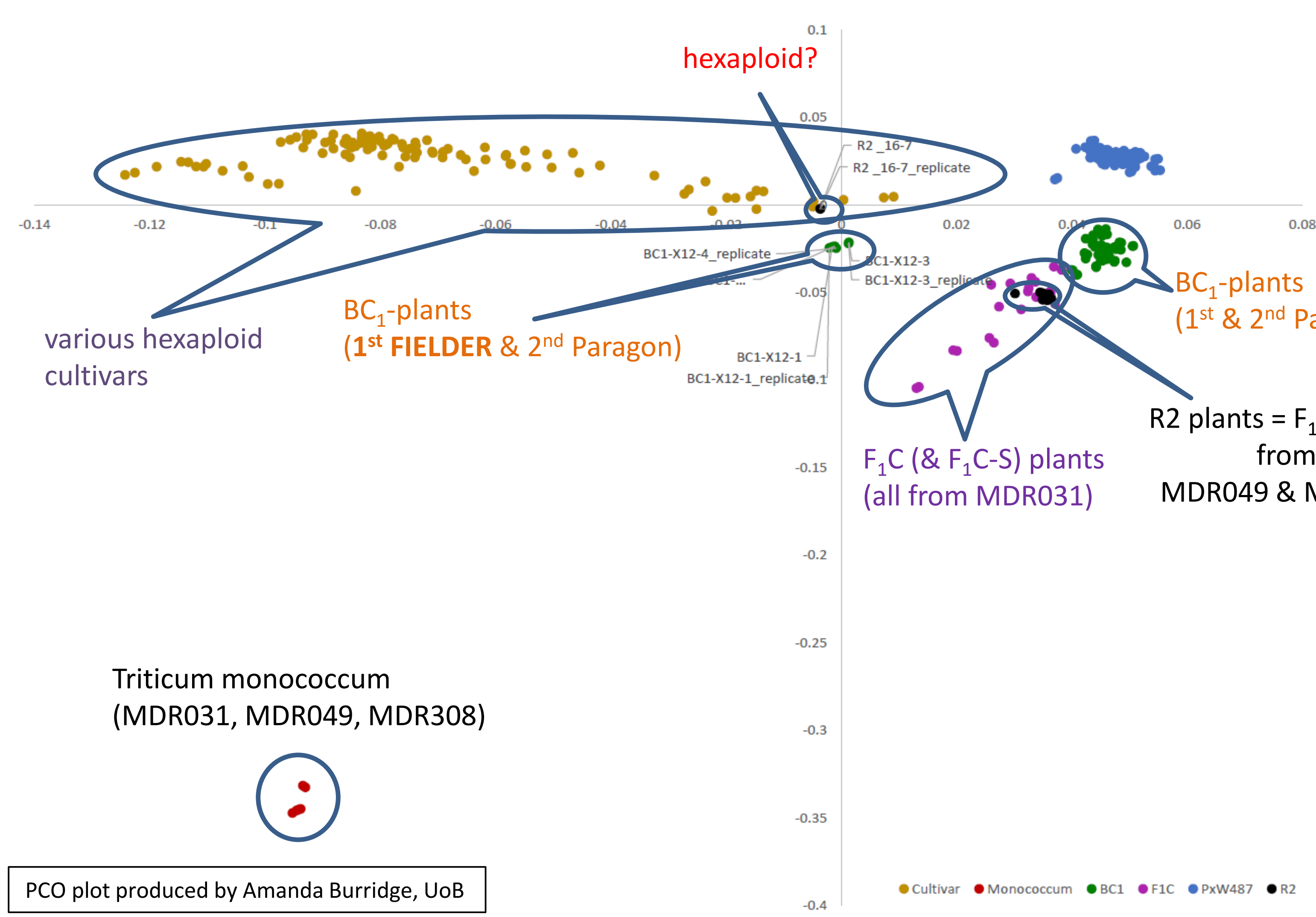

PCO plot produced by Amanda Burridge, UoB

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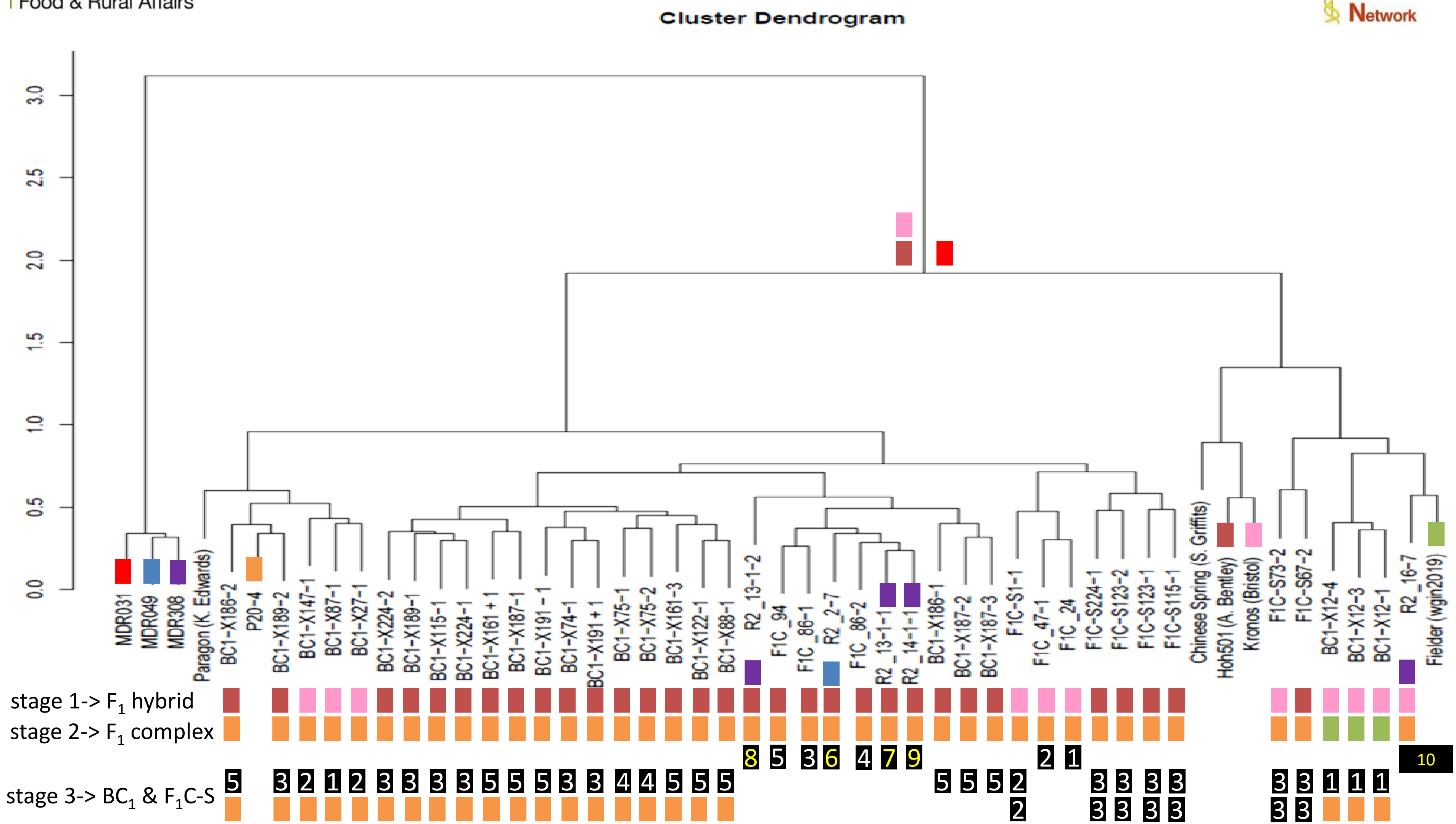

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Similarities of all Parents, F1C, F1C-S and BC1 plants relative to Paragon (P20-4) for the A, B and D-Genomes

A genome

P20-4 (1)	1.00
Paragon	0.92
BC1-X189-2 (1)	0.82
BC1-X186-2 (1)	0.81
BC1-X161-3 (1)	0.80
BC1-X189-1 (1)	0.80
BC1-X87-1	0.80
BC1-X122-1	0.79
BC1-X115-1 (1)	0.79
BC1-X74-1 (1)	0.79
BC1-X88-1 (1)	0.79
BC1-X191 + 1 (1)	0.78
BC1-X147-1 (1)	0.78
BC1-X161 + 1 (1)	0.78
BC1-X75-1 (1)	0.77
BC1-X187-3	0.77
BC1-X27-1 (1)	0.76
BC1-X187-2 (1)	0.76
BC1-X187-1 (1)	0.76
BC1-X224-2 (1)	0.76
BC1-X224-1 (1)	0.75
BC1-X75-2 (1)	0.74
R2 _16-7 (1)	0.74
BC1-X191 - 1 (1)	0.73
F1C-S1-1 (1)	0.73
BC1-X186-1 (1)	0.72
F1C-S123-1 (1)	0.69
F1C-S115-1 (1)	0.68
F1C-S224-1 (1)	0.67
F1C _86-2 (1)	0.66
R2 _13-1-1 (1)	0.66
F1C _47-1 (1)	0.66
R2 _14-1-1 (1)	0.66
F1C _47-1 (2)	0.66
R2 _2-7 (1)	0.66
R2 _13-1-2	0.66
F1C _94 (1)	0.66
F1C _86-1 (1)	0.65
F1C _24 (1)	0.65
F1C-S73-2 (1)	0.65
F1C-S123-2 (1)	0.65
Fielder	0.64
BC1-X12-1 (1)	0.61
BC1-X12-3 (1)	0.61
BC1-X12-4 (1)	0.60
F1C-S67-2 (1)	0.59
Chinese Spring	0.57
Kronos	0.49
Hoh501	0.46
MDR049 (1)	0.28
MDR308 (1)	0.28
MDR031 (1)	0.27

B genome

P20-4 (1)	1.00
Paragon	0.92
BC1-X189-2 (1)	0.81
BC1-X186-2 (1)	0.81
BC1-X161-3 (1)	0.80
BC1-X189-1 (1)	0.80
BC1-X87-1	0.80
BC1-X122-1	0.79
BC1-X115-1 (1)	0.79
BC1-X88-1 (1)	0.79
BC1-X147-1 (1)	0.79
BC1-X74-1 (1)	0.78
BC1-X161 + 1 (1)	0.78
BC1-X191 + 1 (1)	0.78
BC1-X187-3	0.77
BC1-X187-2 (1)	0.77
BC1-X75-1 (1)	0.77
BC1-X27-1 (1)	0.76
BC1-X187-1 (1)	0.76
BC1-X224-2 (1)	0.76
BC1-X224-1 (1)	0.75
R2 _16-7 (1)	0.74
BC1-X75-2 (1)	0.73
BC1-X191 - 1 (1)	0.73
F1C-S1-1 (1)	0.72
BC1-X186-1 (1)	0.72
F1C-S123-1 (1)	0.70
F1C-S115-1 (1)	0.68
F1C-S224-1 (1)	0.67
F1C _47-1 (1)	0.66
R2 _13-1-1 (1)	0.66
F1C _86-2 (1)	0.66
R2 _2-7 (1)	0.65
F1C-S73-2 (1)	0.65
R2 _14-1-1 (1)	0.65
F1C _94 (1)	0.65
R2 _13-1-2	0.65
F1C _86-1 (1)	0.65
F1C _24 (1)	0.65
Fielder	0.65
F1C-S123-2 (1)	0.64
BC1-X12-1 (1)	0.61
BC1-X12-3 (1)	0.60
F1C-S67-2 (1)	0.60
BC1-X12-4 (1)	0.60
Chinese Spring	0.56
Kronos	0.50
Hoh501	0.46
MDR308 (1)	0.28
MDR049 (1)	0.28
MDR031 (1)	0.27

D genome

P20-4	1.00
Paragon	0.92
BC1-X189-2	0.81
BC1-X87-1	0.81
BC1-X186-2	0.81
BC1-X161-3	0.80
BC1-X189-1	0.80
BC1-X122-1	0.80
BC1-X115-1	0.79
BC1-X74-1	0.79
BC1-X88-1	0.79
BC1-X147-1	0.79
BC1-X191 + 1	0.78
BC1-X161 + 1	0.78
BC1-X187-2	0.77
BC1-X27-1	0.77
BC1-X187-3	0.77
BC1-X75-1	0.76
BC1-X224-2	0.76
BC1-X187-1	0.76
BC1-X224-1	0.75
BC1-X75-2	0.74
R2 _16-7	0.73
BC1-X191 - 1	0.73
F1C-S1-1	0.73
BC1-X186-1	0.72
F1C-S123-1	0.69
F1C-S115-1	0.68
F1C-S224-1	0.67
R2 _13-1-1	0.66
F1C _47-1	0.66
F1C _86-2	0.66
R2 _14-1-1	0.66
F1C _94	0.66
R2 _2-7	0.66
F1C _24	0.65
R2 _13-1-2	0.65
F1C _86-1	0.65
Fielder	0.65
F1C-S73-2	0.65
F1C-S123-2	0.64
BC1-X12-1	0.61
BC1-X12-3	0.61
BC1-X12-4	0.60
F1C-S67-2	0.59
Chinese Spring	0.56
Kronos	0.49
Hoh501	0.45
MDR049	0.28
MDR308	0.28
MDR031	0.28

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What's Next?

1. Axiom Analysis – ongoing, help please.
2. Generation of BC_3 grain from BC_2 plants – 188 germinated grains planted yesterday
3. Zymoseptoria resistance tests – ongoing (with Kostya), leaf inocculations last Friday -> initial results in 2-3 weeks
4. Genotyping By Sequencing – collaboration with Dragan Perovic (Julius Kuehn-Institute, DE)

1.

2.

3.

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4.

frontiers in Plant Science

ORIGINAL RESEARCH
published: 24 September 2019
doi: 10.3389/fpls.2019.01133

Check for updates

Detecting Large Chromosomal Modifications Using Short Read Data From Genotyping-by-Sequencing

Jens Keilwagen[1], Heike Lehnert[1], Thomas Berner[1], Sebastian Beier[2], Uwe Scholz[2], Axel Himmelbach[3], Nils Stein[3], Ekaterina D. Badaeva[4], Daniel Lang[5], Benjamin Kilian[6], Bernd Hackauf[7] and Dragan Perovic[8]*

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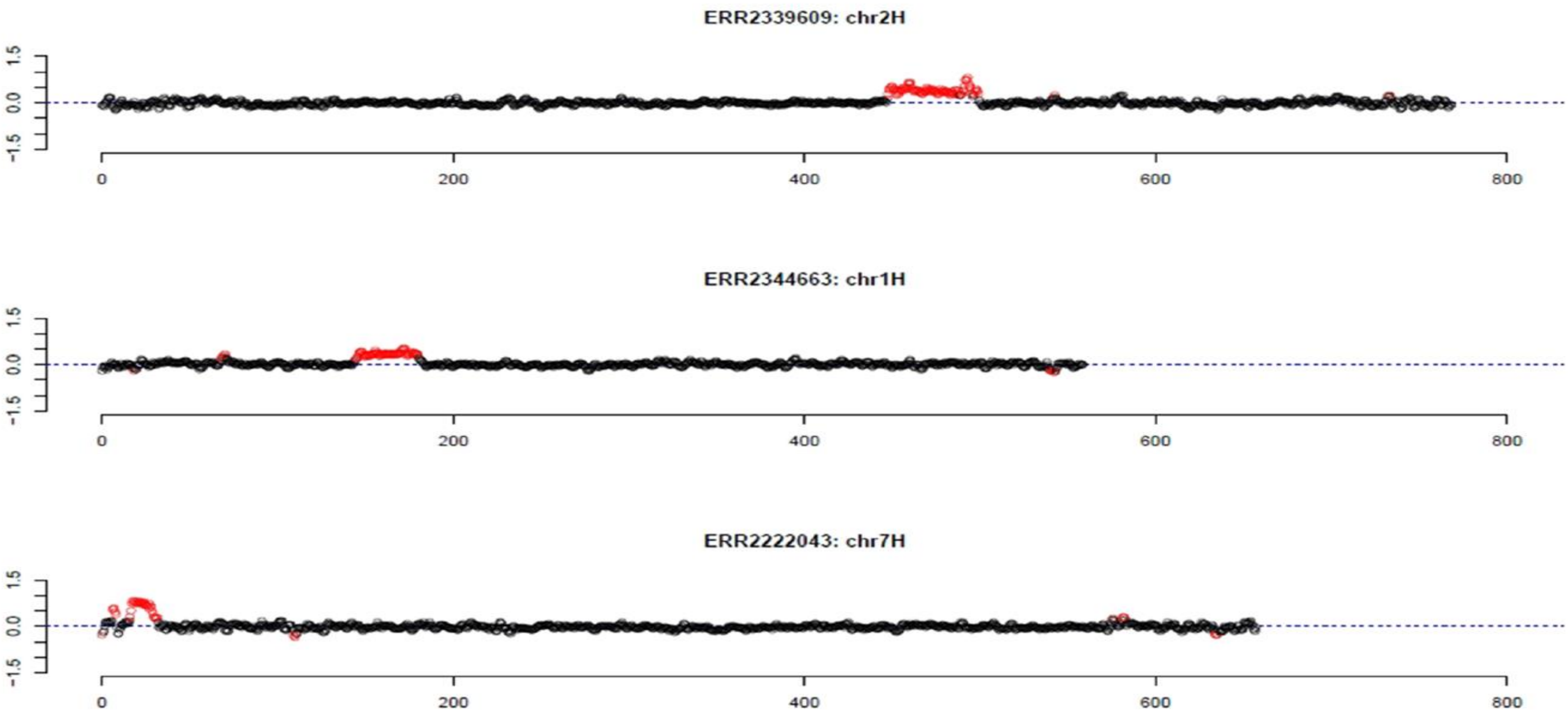

FIGURE 3 | Outliers of GBS coverage data for barley collection of the German Genebank. The x-axis depicts the position within the chromosome in Mb, while the y-axis depicts normalized coverage. Each dot visualizes the denoised coverage value of a non-overlapping 500 kb window, while the dashed line depicts the expectation. Dots are depicted in red if they are marked as outliers indicating large chromosomal modifications.

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E

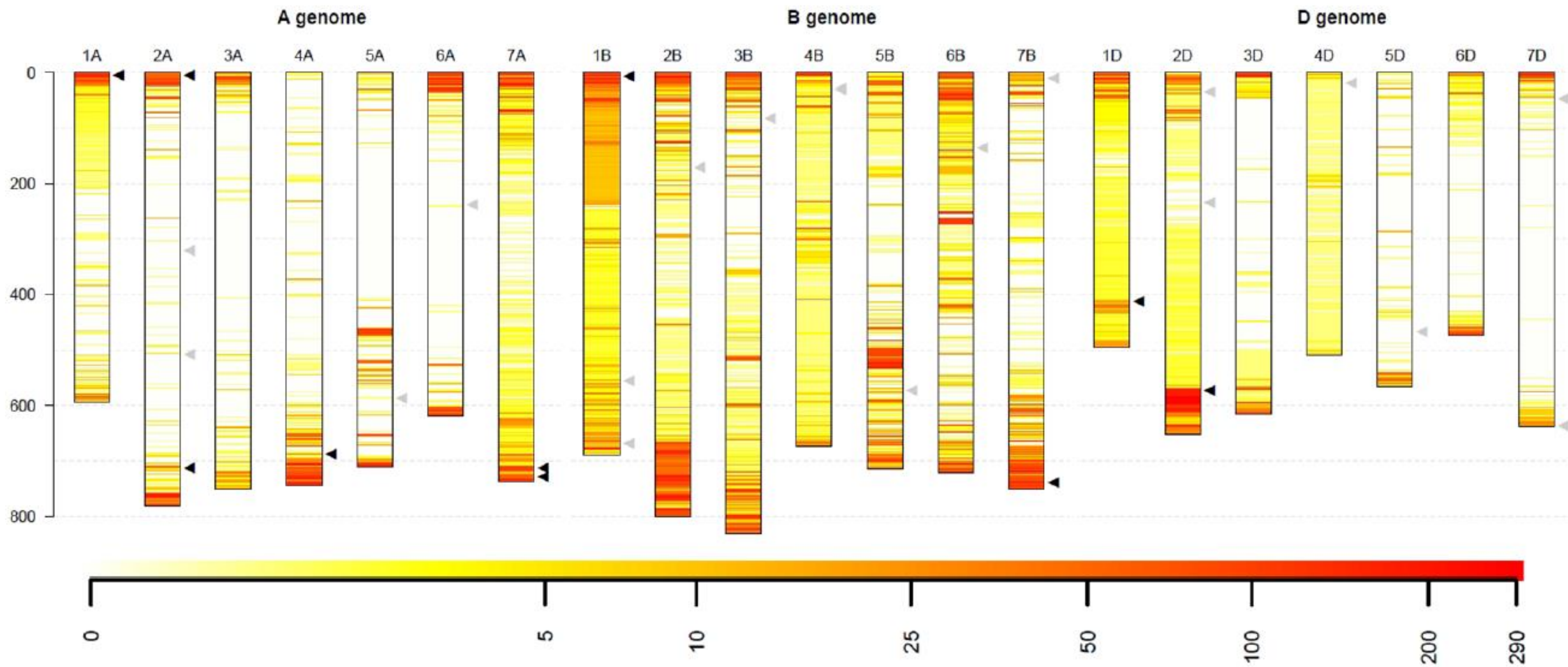


FIGURE 6 | Statistics of outliers per genome and chromosome for the winter wheat collection comprising 290 genotypes. **(A)** and **(B)** show the statistics for all outliers, while **(C)** and **(D)** depict the outliers that were detected in at least 10% of the genotypes. **(E)** depicts the spatial distribution of the outliers along the chromosomes (in Mb) and their frequency. Heat colors are used to visualize the frequency of outliers on a logarithmic scale where white indicates no wheat genotype with an outlier and red indicates many wheat genotypes with an outlier at this locus. Triangles indicate genes with interest for breeding. Black triangles indicate genes that are located in regions with many outliers within the collection, while gray triangles indicate genes in regions with a low number of outliers.

Rothamsted Research
where knowledge grows

Resilience to foliar and root fungal pathogens

Kim Hammond-Kosack
Vanessa McMillan
Gail Canning

Wheat Genetic Improvement Network

Department for Environment Food & Rural Affairs

WGIN MM 8th Oct 2019

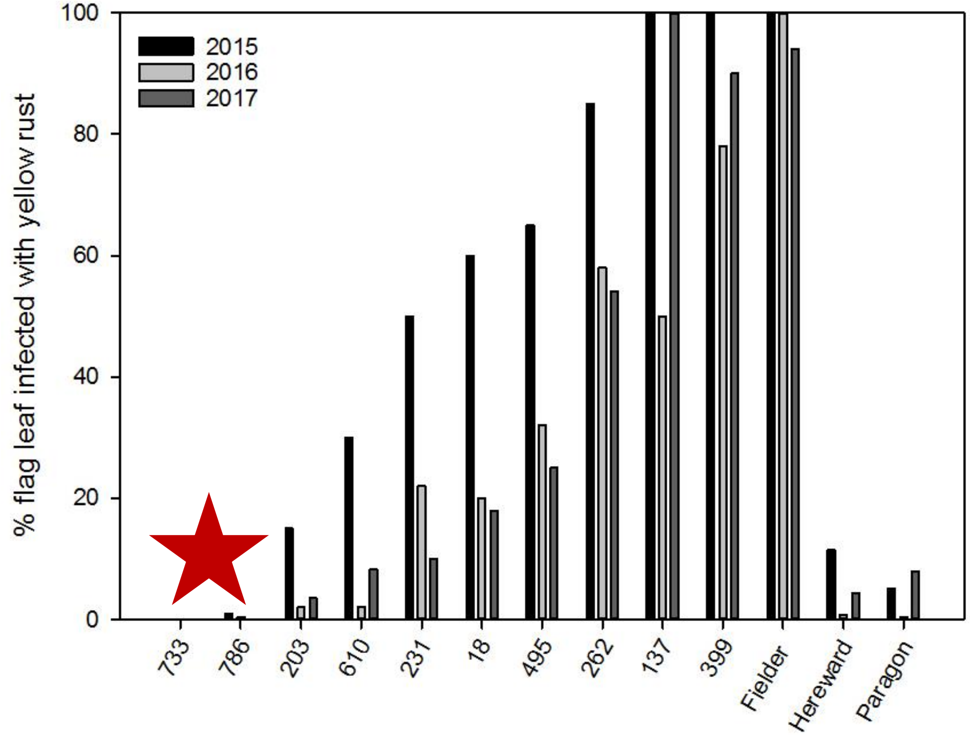
BBSRC bioscience for the future

ROTHAMSTED RESEARCH

WGIN 4 objectives

- Resistance to yellow rust
- *mlo* mediated resistance to powdery mildew
- 3N ancestral introgression rooting trait and improved resistance to Take-all

Evidence of resistance to yellow rust



Adult plant resistance in field trials

- **Five Watkins genotypes with moderate/strong resistance against yellow rust (WGIN 3)**
- **All fully susceptible at the seedling stage (isolates Purple3, Blue 7, Red 24 NIAB, 2018)**

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

2018/19 Field Trial

F2 and F3 families phenotyped to identify homozygous susceptible and resistant lines plus retest individual F1 plants

Evidence of resistance to yellow rust

ROTHAMSTED RESEARCH

2018/19 Field Trial

F2 and F3 families phenotyped to identify homozygous susceptible and resistant lines plus retest individual F1 plants (n = 6 for each)

Adult plant resistance in field trials

F1 W733 x Fielder = Fully resistant (Dominant trait)

F1 W786 x Fielder = Fully susceptible (Recessive trait)

Note : Yellow rust levels were very high on the RRes farm

F2 W733 x Fielder : 53 resistant plants : 38 susceptible plants

68 : 23 (1 locus) predicted

F3 W733 x Fielder 11 lines all 80 plants resistant

3 lines all 80 plants susceptible

23 lines segregating

Options going forward

- **Bulk segregant analysis plus 10 F2 S lines field phenotypes 2018/19**
- **Wheat 35K array analysis of individual lines**
- **REN Seq for NBS-LRRs type of *R* genes with Brand Wulff**

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Evidence of resistance to yellow rust

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2018/19 Field Trial

F2 and F3 families will be phenotyped to identify homozygous susceptible and resistant lines plus retest individual F1 plants (n = 6 for each)

Adult plant resistance in field trials

F1 W786 x Fielder = Fully susceptible (Recessive trait)

F2 W786 x Fielder : 107 susceptible plants

: 27 host response, no sporulation

101 : 33 (1 locus) predicted

F3 W786 x Fielder

1 lines all 80 plants resistant - seed

11 lines all 80 plants fully susceptible – seed

36 lines all 80 plants with some YR

17 lines segregating → 190 plants collected seed to re-phenotype in 2019/20

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WGIN 4 objectives

- Resistance to yellow rust
- ***mlo* mediated resistance to powdery mildew**
- 3N ancestral introgression rooting trait and improved resistance to Take-all

Powdery Mildew

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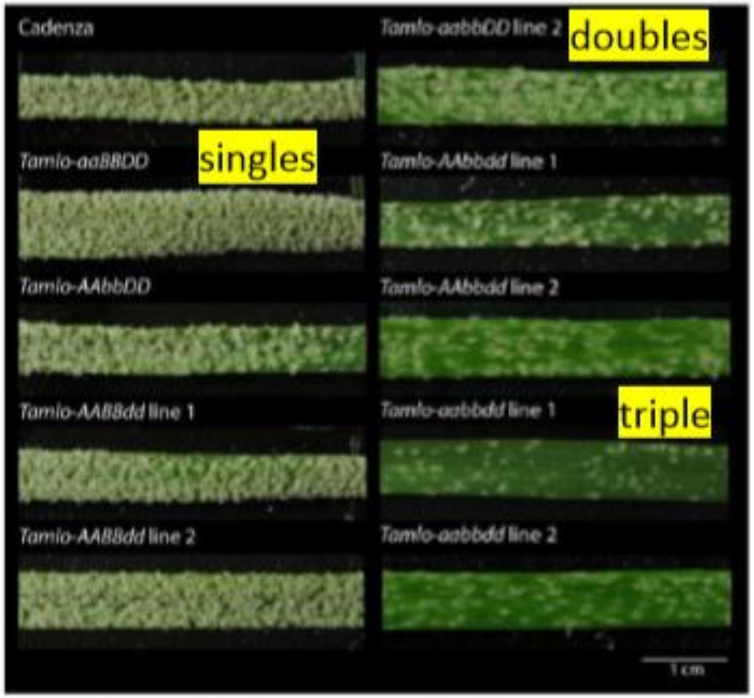
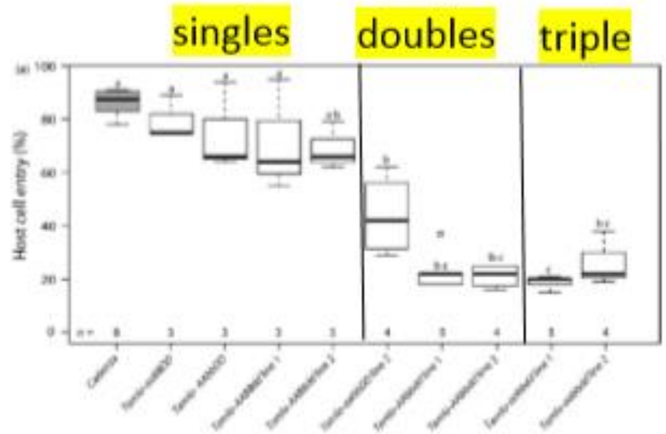
TILLING wheat for *mlo* mediated mildew resistance





Seedling glasshouse screen

Cv Cadenza

Initially WGIN funded

Plant Biotechnology Journal 2017

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

Johanna Acevedo-Garcia[1], David Spencer[1], Hannah Thieron[1], Anja Reinstädler[1], Kim Hammond-Kosack[2], Andrew L. Phillips[2] and Ralph Panstruga[1]*

Funders: German Federal Ministry of Food and Agriculture

Germany Society for the Advancement of Plant Innovation

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Are there trade offs under field conditions?

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2017/18 Spring sown multiplication field trial (results presented Oct 2018 MM)

Summary

- 18 selected double and triple mutants (single replicate, 30 seeds)
- Replicated plots of Cadenza wildtype (Source seed of TILLING population)
- Foliar disease observations - Trend for a reduction in powdery mildew
- Some doubles and triples with higher yellow rust infection

- Ear emergence - Trend WT Cadenza earlier into flowering than *mlo* mutants
- Plant heights - Trend WT Cadenza taller than *mlo* mutants
- Senescence - Trend leaf 2 WT Cadenza senesced earlier than *mlo* mutants

mlo mediated resistance

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

- Plant height, ear emergence, leaf senescence (top 4 leaves)

Fusarium – spray inoculation at anthesis with overnight bagging

Results so far

4 lines poorly established after which all the plants died due to winter conditions (Note - field seed bulking successful from a spring sowing)

Yellow rust data - good level of disease

Fusarium data – scored 20 ears/plot at 21 days post inoculation

Septoria data – none even with regular irrigation + spreader line

All data with statisticans

Repeat trial 2019/2020 with the Septoria trial in North Devon

WGIN 4 objectives

- Resistance to yellow rust
- *mlo* mediated resistance to powdery mildew
- 3N ancestral introgression rooting trait and improved resistance to Take-all

3N introgression mediated resistance to Take-all

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Background

The 3N chromosome segment from *Aegilops uniaristata* has been introgressed into Chinese Spring

From the literature chromosome 3N has been shown to confer tolerance to aluminium toxicity in contaminated soils via prolific root development

Three substitution lines were generated in the late 1990s in which 3N replaces the homoeologous wheat chromosomes, 3A, 3B or 3D.

Miller …. King IP New Phytol. (1997), 137, 93-98

By using the *ph-1* pairing locus mutant a smaller alien segment which carries the gene(s) for tolerance but not the agronomically unacceptable brittle rachis gene also carried on chromosome 3N. JIC

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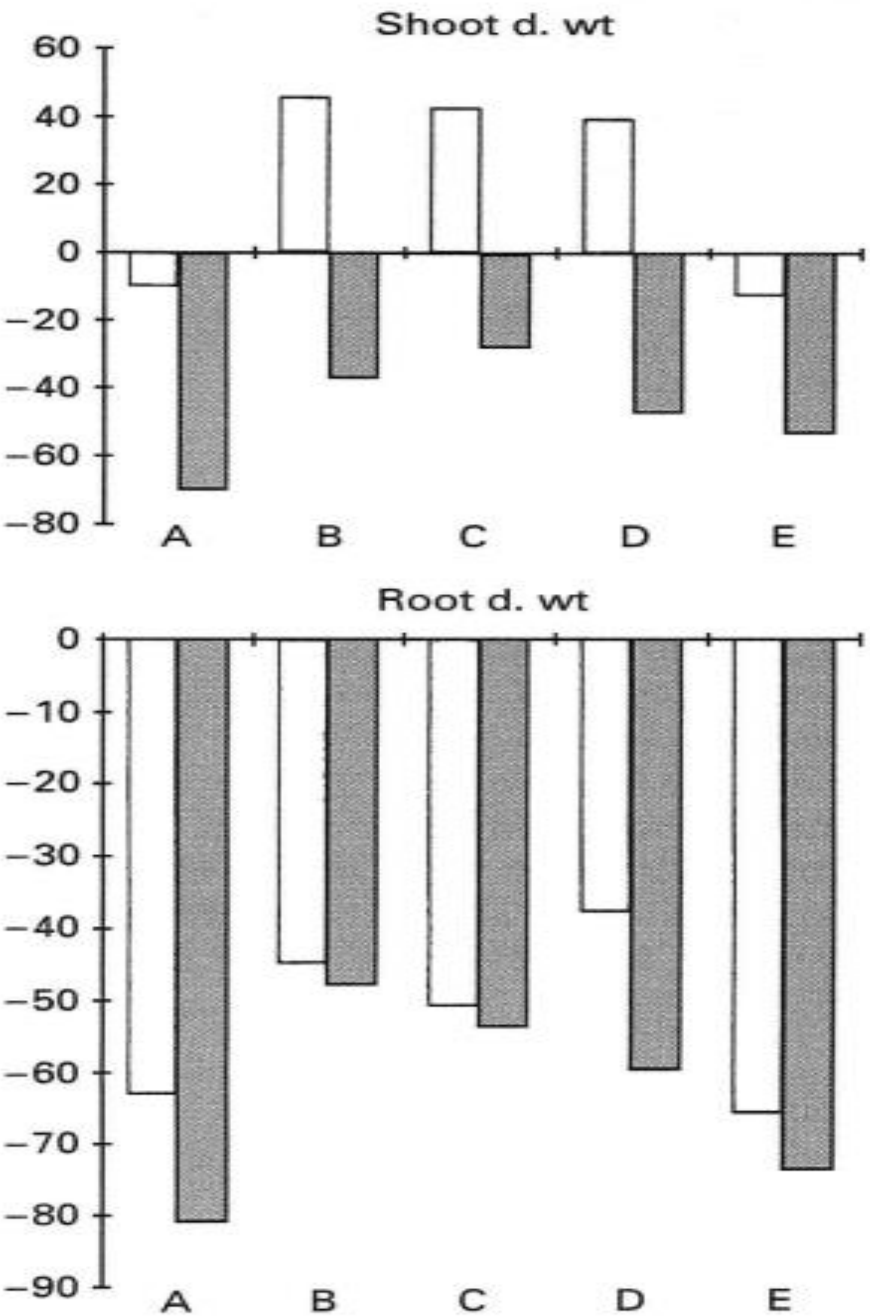

Data from : Miller …. King IP New Phytol. (1997), 137, 93-98

Figure 3. Mean effects on a range of plant characters 20 ppm Al (white bars)
40 ppm Al (grey bars)

A Chinese Spring (control)
B CS/**3N addition**
C CS(3A)**3N substitution**;
D CS(3D)**3N substitution**;
E Brazilian cv. BH1146.

The data are expressed as the percentage difference from the control treatment (0 ppm Al).

3N introgression mediated resistance to Take-all

ROTHAMSTED RESEARCH

The 3N chromosome segment in Chinese Spring background on take-all resistance

Working hypotheses

1. Overall a larger root biomass under 'stressful' conditions – abiotic or biotic

2. The 3N introgressed segment confers a general re-rooting response under biotic or abiotic stresses

Progress of far - Initial seed multiplication – completed in glasshouse

Take-all experiments (planned)

Seedling pot test for 5 weeks with / without the take-all fungus – assess root systems for disease severity / incidence and root development

Continue the infected plants + Amistar treatment for further seed production in GH

Spring 2020

Large pot tests in screenhouse - with / without the take-all fungus

Further seed multiplication

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Many thanks to

Gail Canning
Jessica Hammond (Plant Pathology Apprentice)
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Kostya Kanyuka
Mike Hammond-Kosack
Carlos Bayon
Tania Chancellor (2nd year PhD student)

RRes Farm and glasshouse staff

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& 2019)
Georgie Halford (2018)
Eoin Canning (2018 & 2019)
Niamh Kavanagh (2018)

Rodger White, Stephen Powers and Suzanne Clark - statistics

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