Wheat
Genetic
Improvement
Network

John Innes Centre

Gene Content around QTL loci in Avalon x Cadenza NILs

Clare Lister and Simon Griffiths
(Sue Freeman, Alba Farre-Martinez and Ania Kowalski
and earlier members of Griffiths group)

08/05/2019

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QTL loci

- The QTLs were identified in NILs derived from the AxC DH population and had been mapped originally using mainly microsatellite markers
- Height 2D
- Height and Flowering 3A
- Flowering 6A

- Previous work from Resilience and RUE LINK projects and AK PhD project*

*(The Agronomic and Molecular Characterisation of *Rht8* in Hexaploid Wheat)

QTL loci

- Mapping with KASP refined the QTL positions
- Height and Flowering 3A
- QTL mapping for both traits (AFM/SF/CL)

- Height 2D
- Locus finely mapped (AK)

- Flowering 6A
- Mapping (SF/CL)

Stategy for determining Gene Content

- The position of the KASP markers flanking the QTLs region located in the IWGSC RefSeq v1.0 sequence
- All coding sequences within region downloaded from EnsemblPlants using BioMart.
- The sequences analysed using BLASTX at NCBI against database of **non-redundant higher plant protein** sequences.
- In all instances homologous sequences were detected, but gene identity was not always defined.

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3A Height and DTEM QTLs

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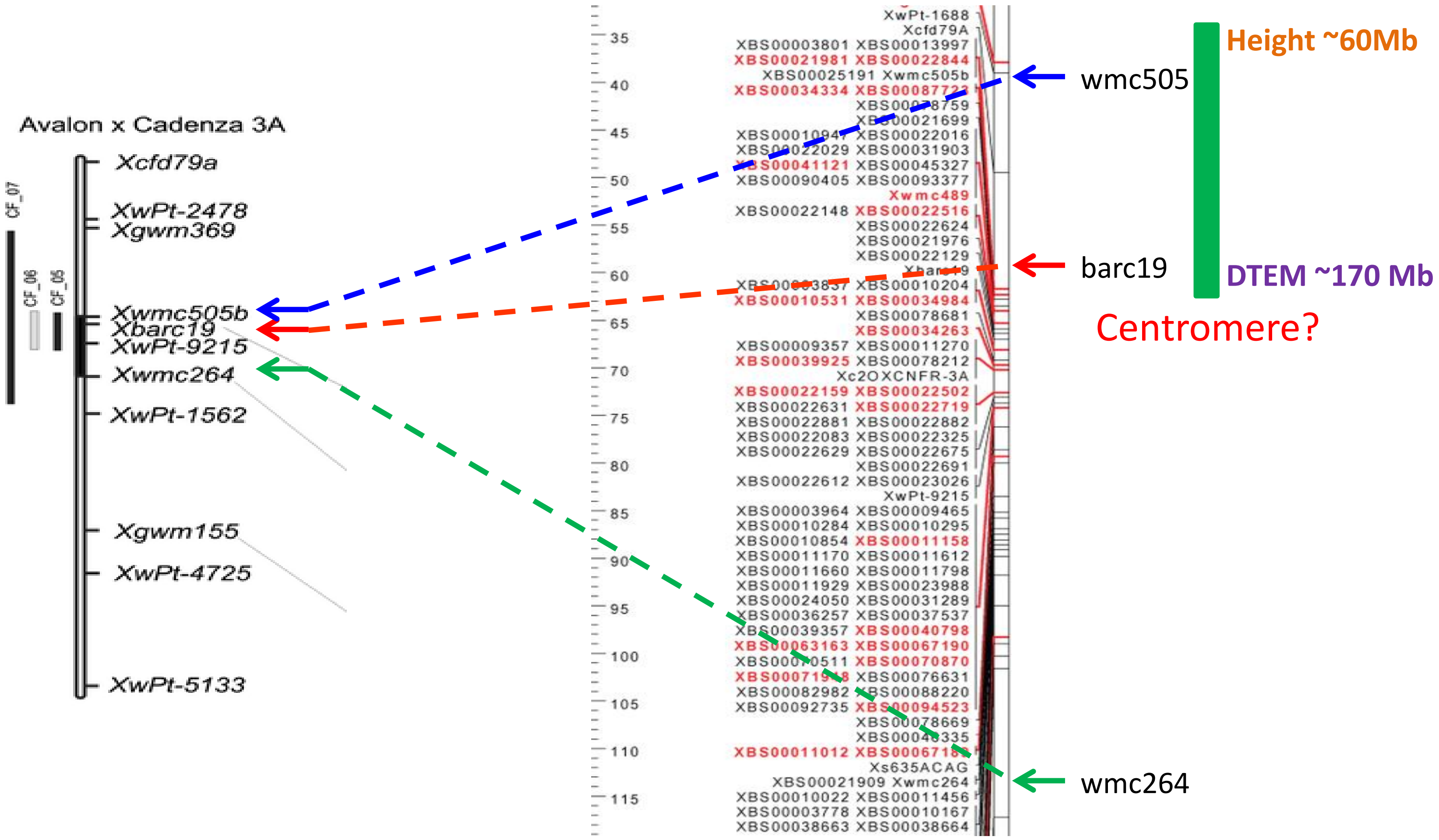

wmc505

barc19

wmc264

Height ~60Mb

DTEM ~170 Mb

Centromere?

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3A HT and DTEM

- Two QTLs on Chr 3AS, one for height and the other flowering
- Possibility of third gene affecting yield between them.
- Large region of sequence analysed (45 – 210 Mb).
- Additionally had RNA seq data (early + short / late + tall pools) – recently re-analysed.
- Over 1000 genes are located in region
- Putative gene candidates for flowering and especially height identified and being investigated.

3A HT and DTEM Candidates

Gene ID	Start	Mapping Marker	CLEAR SNPs Ava v Cadenza	high expression diff	ID
TraesCS3A02G092900	58927199		x		PREDICTED: vegetative cell wall protein gp1-like
TraesCS3A02G093400	59822956		int and ex		xyloglucan endotransglycosylase/hydrolase protein 8-like
TraesCS3A02G093500	59954598		int and ex	▨	xyloglucan endotransglycosylase/hydrolase protein 8-like
TraesCS3A02G093600	60005281		x		xyloglucan endotransglycosylase/hydrolase protein 8-like
TraesCS3A02G093700	60028751		ex	▨	Xyloglucan endotransglycosylase/hydrolase protein 8
TraesCS3A02G093800	60193220		ex		60.2 Mb xyloglucan galactosyltransferase KATAMARI1 homolog
TraesCS3A02G094000	60203935		x	▨	glycine-rich cell wall structural protein-like
TraesCS3A02G094600	60446831		int and ex		Cellulose synthase-like protein G2
TraesCS3A02G096000	61311578		int and ex?	▨	amino-acid permease BAT1 homolog
TraesCS3A02G096200	61340198	BS00003801	ex		protein NETWORKED 1A
TraesCS3A02G096300	61346177	BS00022516 / BS00013997	int and ex?		protein NETWORKED 1A
TraesCS3A02G099400	64278873		x	▨	serine/threonine-protein kinase BRI1-like 2
TraesCS3A02G100500	65360869		ex		non-classical arabinogalactan protein 30-like
TraesCS3A02G116300	84184377		x		TaGI
TraesCS3A02G122600	97972874		x	▨	Gibberellin 3-beta-dioxygenase 2-1
TraesCS3A02G133400	110332577		x	▨	gibberellin 2-beta-dioxygenase 1-like
TraesCS3A02G136500	114041077		x		SHAGGY-like kinase (related to AtBIN2)
TraesCS3A02G143100	124172881		x	▨	FT-like protein (FT2)
TraesCS3A02G155200	147789276		5'?		IAA3
TraesCS3A02G156500	151642540		x		ABI8
TraesCS3A02G156600	151675660		x	▨	auxin response factor 1 isoform X3 (but v short homologous region)
TraesCS3A02G159200	158467595		x		ARF1
?	158739408		?	?	protein FAR1-RELATED SEQUENCE 5-like
?	167873821		?	?	protein FAR1-RELATED SEQUENCE 5-like
TraesCS3A02G164200	168837089	BS00021976	ex and 3'		shaggy-related protein kinase alpha
TraesCS3A02G167100	172629517		x		putative brassinosteroid receptor
TraesCS3A02G173300	191527521		x		Topless
TraesCS3A02G176100	196761940		x	▨	flowering-promoting factor 1-like protein 1
TraesCS3A02G180600	208246099		x		cyclic dof factor (CDF) 2-like

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6A DTEM

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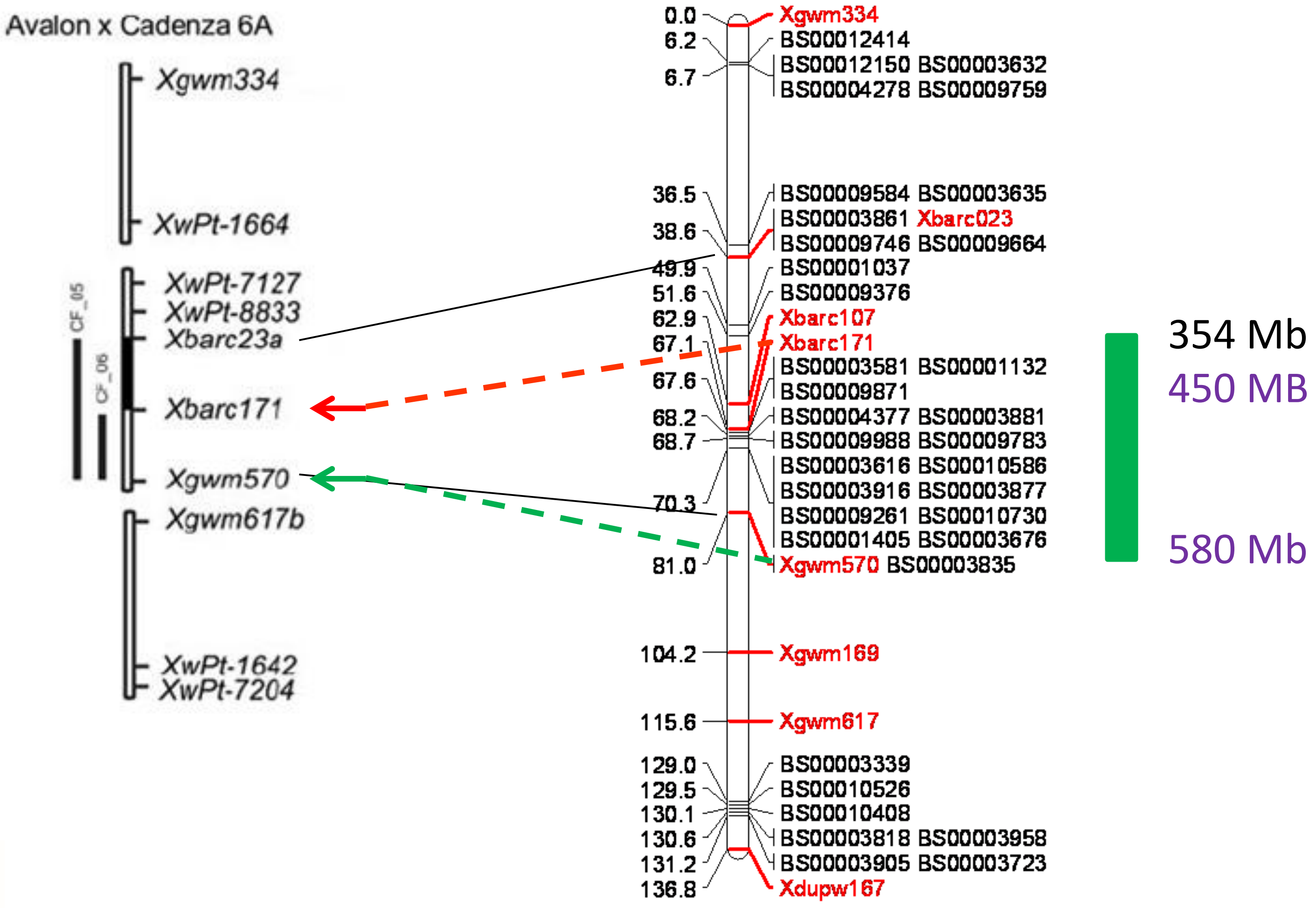
6A SNP map



354 Mb
450 MB
580 Mb

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6A DTEM

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- The flowering QTL on Chr 6A likely to be in region of approx 130 Mb (450 - 580 Mb)
- This region contains approximately 1050 genes
- A number of putative gene candidates which might be involved in flowering have been identified.

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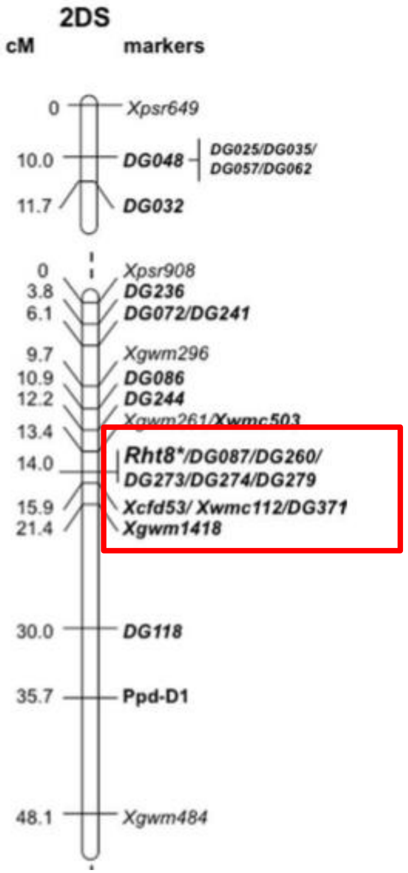
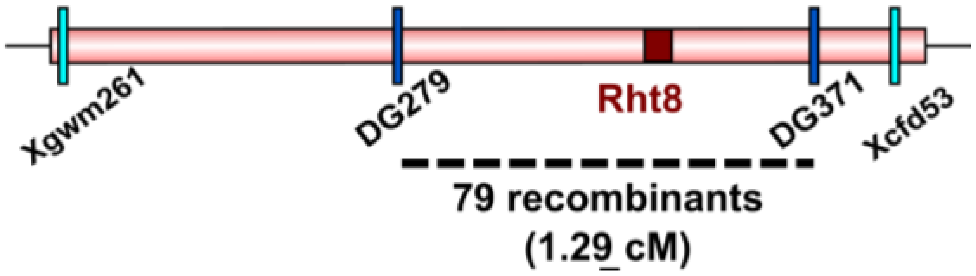
6A DTEM

Gene ID	Start	ID
>TraesCS6A02G246200	457370874	protein SPT2 homolog
>TraesCS6A02G246700	457617229	FT-interacting protein 1-like
>TraesCS6A02G247200	459074425	scarecrow-like protein 6
>TraesCS6A02G251200	465605228	E3 ubiquitin-protein ligase BRE1-like 1 isoform X2
>TraesCS6A02G254600	471867051	protein argonaute 1A-like isoform X2
>TraesCS6A02G262200	485377091	BOI-related E3 ubiquitin-protein ligase 1-like
>TraesCS6A02G268800	495444913	transcription factor EAT1-like
>TraesCS6A02G286400	518666410	zinc finger protein CONSTANS-LIKE 10-like isoform X2
>TraesCS6A02G293200	524718099	zinc finger protein CONSTANS-LIKE 16-like
>TraesCS6A02G296900	530251497	Gibberellin 3-beta-dioxygenase 1
>TraesCS6A02G306600	540601341	Cullin-3A
>TraesCS6A02G313800	550637111	MADS-box transcription factor TaAGL11
>TraesCS6A02G316600	553003172	protein EXORDIUM-like
>TraesCS6A02G318300	554459596	lysine-specific histone demethylase 1 homolog 1 = FLD
>TraesCS6A02G326100	559421439	E3 ubiquitin-protein ligase COP1
>TraesCS6A02G333600	564715783	ABI5
>TraesCS6A02G336900	570939841	protein FIP1-like isoform X1
>TraesCS6A02G338000	571795464	protein BZR1 homolog 3-like, partial
>TraesCS6A02G338400	571881423	agamous-like MADS-box protein AGL29
>TraesCS6A02G343500	577747167	protein TONSOKU
>TraesCS6A02G344800	578895724	Serine/threonine-protein kinase CTR1
>TraesCS6A02G347500	579952353	protein FLX-like 1

2D HT = *Rht8*





DG279	SSCP	22201719
52i	KASP	22203609
2DS_5375260	SSR	22446675
55_uni	KASP	22451012
Marker 16_uni	KASP	24944815
DG371	SSCP	24975783

2D HT = *Rht8*

- As very fine mapping has already been carried out for the height QTL on Chr 2D a region of less than 3 Mb was analysed.
- There are around 30 genes in this region
- There are two possible candidates

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Final comments

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- IWGSC RefSeq v1.0 sequence – indispensble!

- …but is one variety, Chinese Spring.

- Other varieties may be rearranged compared to CS?
- i.e. 3A DTEM – QTL mapping position v sequence position

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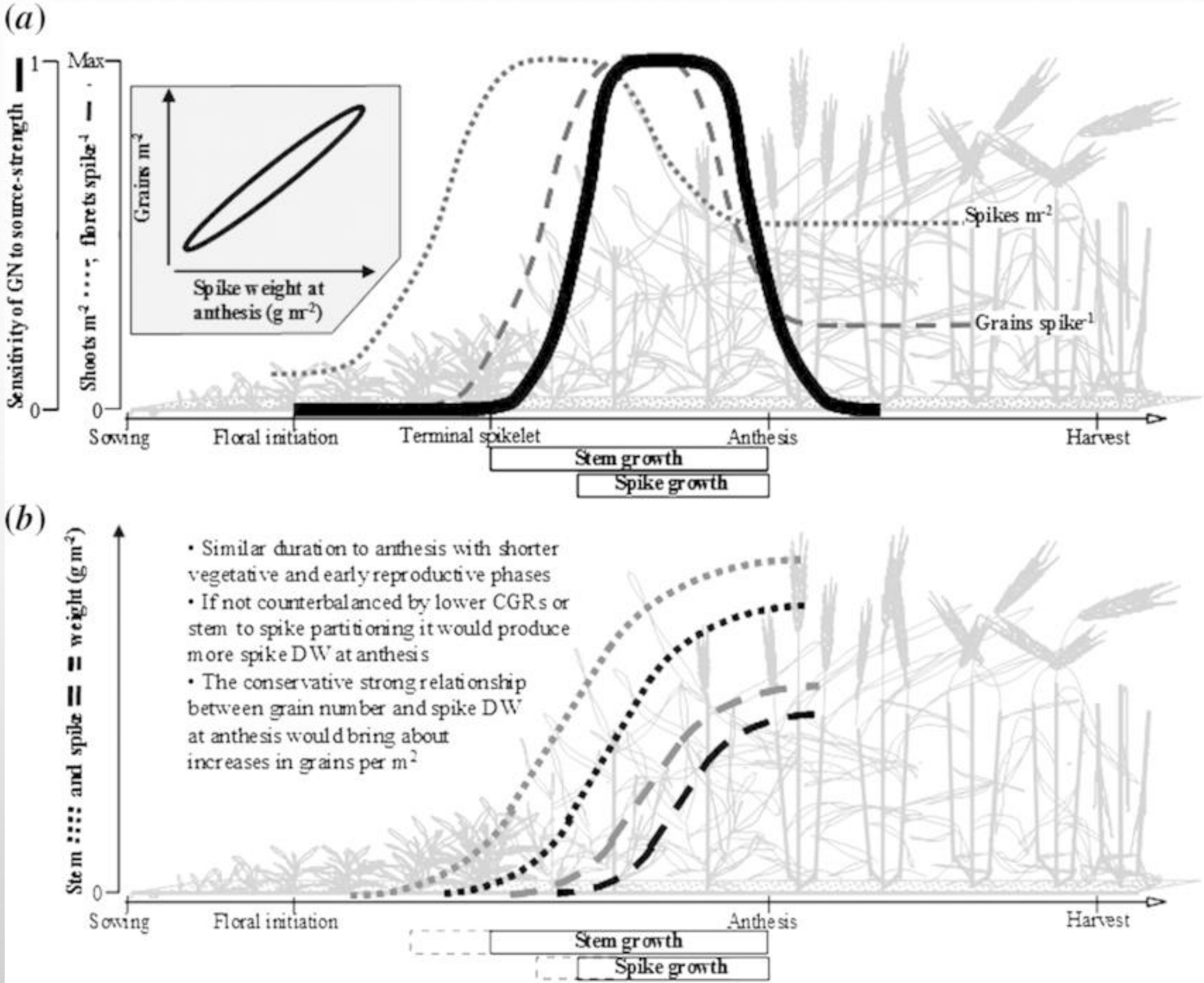
JIC PRESENTATION FOR WGIN MANAGEMENT

KWS 8TH MAY 2019

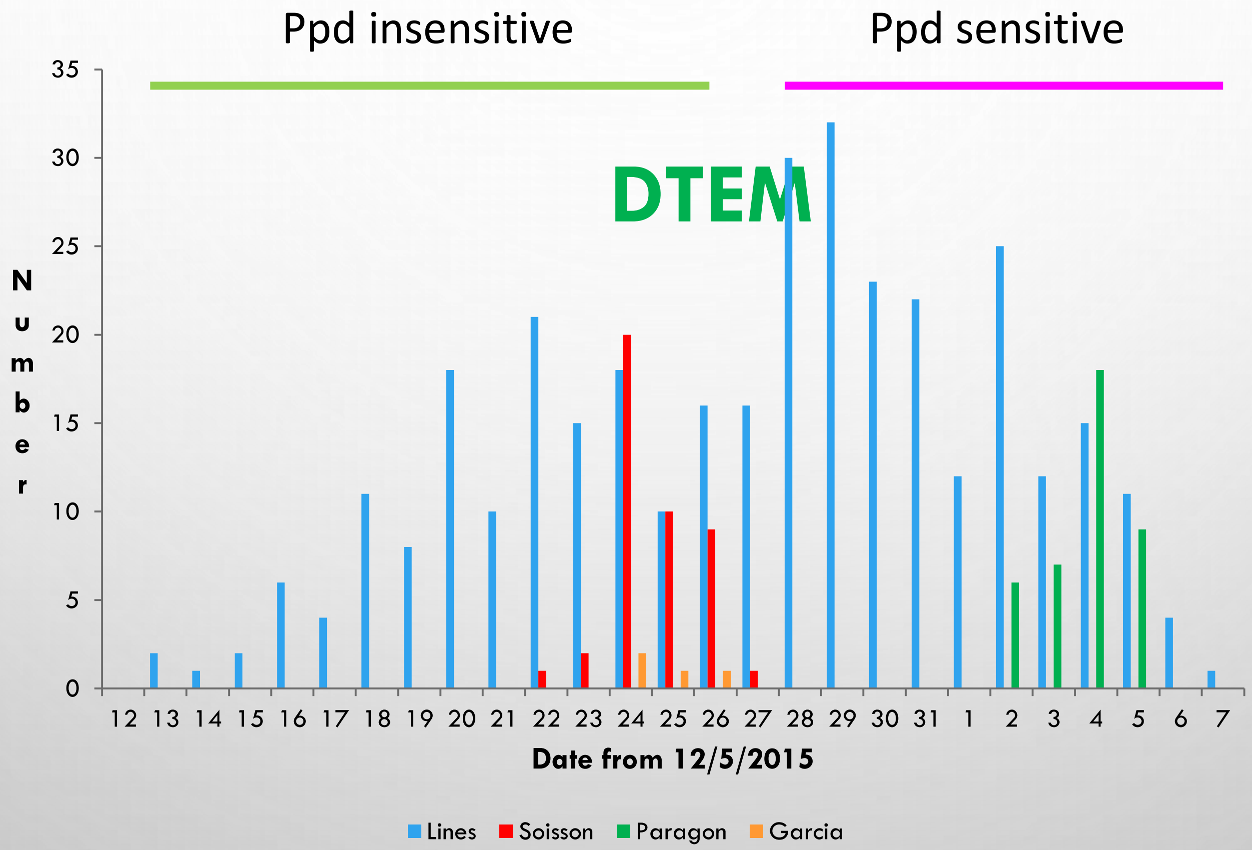
DROUGHT STUDY UPDATE

SPRING DROUGHT COINCIDES WITH GRAIN NUMBER DETERMINATION



- Similar duration to anthesis with shorter vegetative and early reproductive phases
- If not counterbalanced by lower CGRs or stem to spike partitioning it would produce more spike DW at anthesis
- The conservative strong relationship between grain number and spike DW at anthesis would bring about increases in grains per m^2

Reducing early flowering escapes



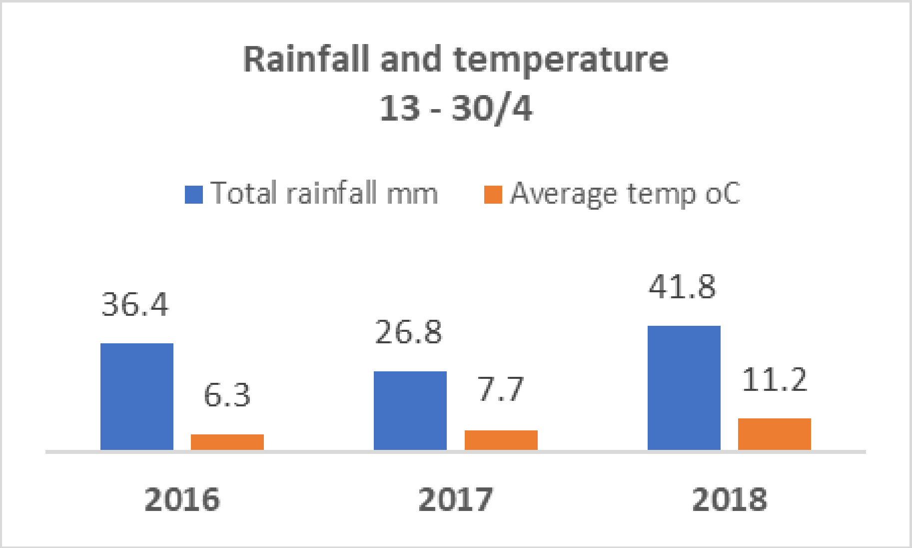
177 RILs in UK adapted window

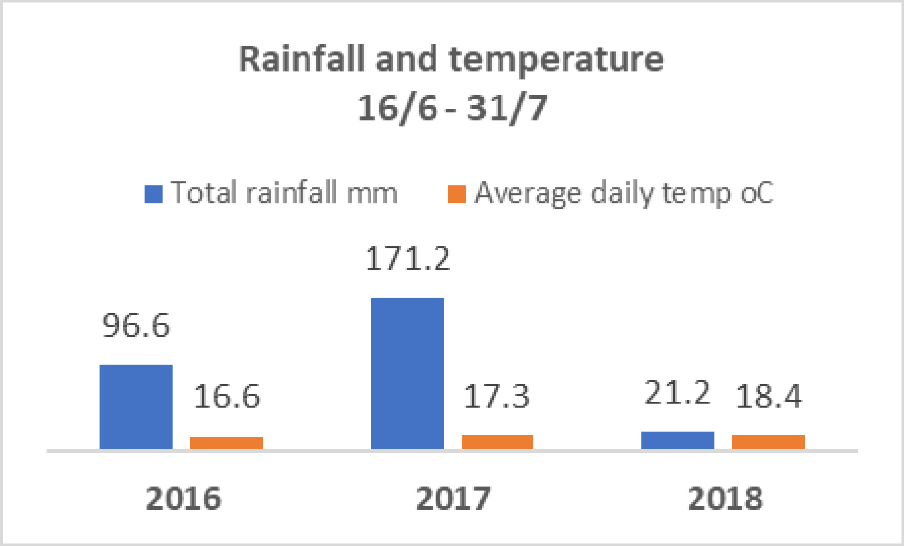
Control and certainty

RAIN-OUT SHELTERS VERSUS +/- IRRIGATION

Flexibility and scalability

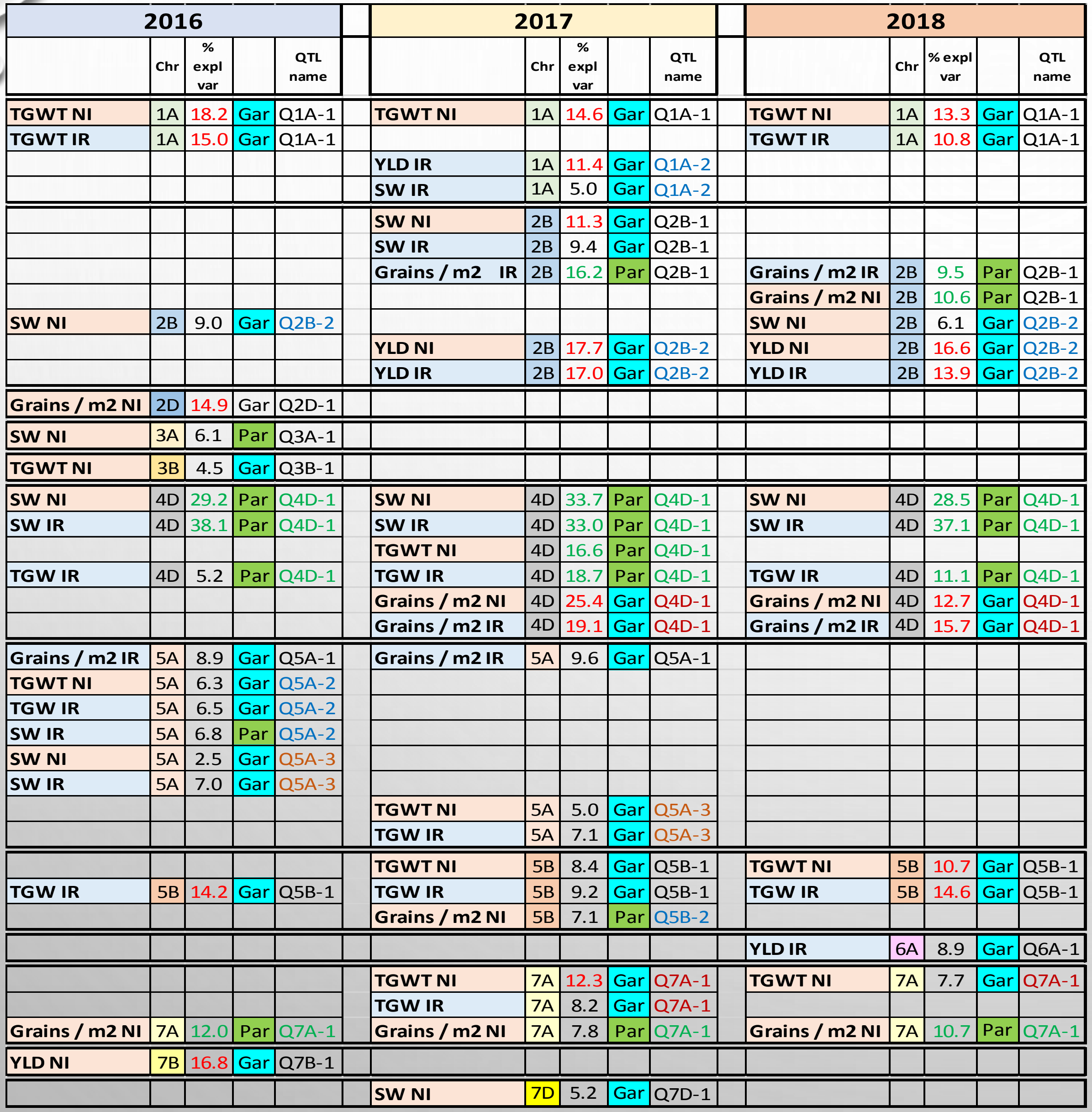
WEATHER DURING WGIN DROUGHT EXPERIMENTS SO FAR





2019?

Summary of QTL identified so far

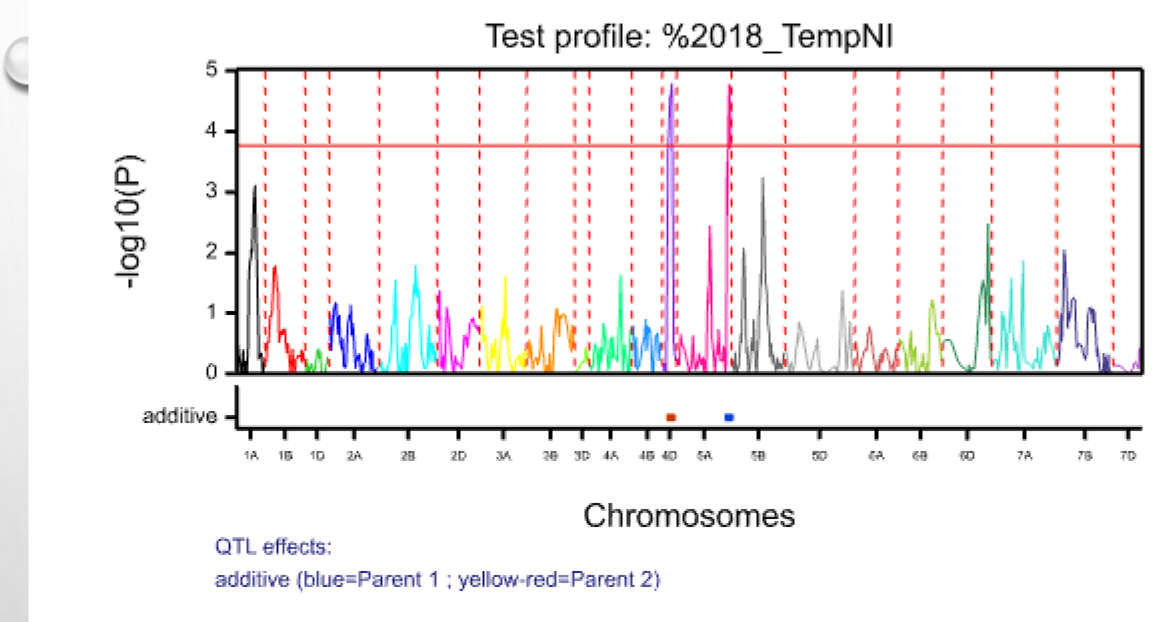


2016					2017					2018				
	Chr	% expl var		QTL name		Chr	% expl var		QTL name		Chr	% expl var		QTL name
TGWT NI	1A	18.2	Gar	Q1A-1	TGWT NI	1A	14.6	Gar	Q1A-1	TGWT NI	1A	13.3	Gar	Q1A-1
TGWT IR	1A	15.0	Gar	Q1A-1						TGWT IR	1A	10.8	Gar	Q1A-1
					YLD IR	1A	11.4	Gar	Q1A-2					
					SW IR	1A	5.0	Gar	Q1A-2					
					SW NI	2B	11.3	Gar	Q2B-1					
					SW IR	2B	9.4	Gar	Q2B-1					
					Grains / m2 IR	2B	16.2	Par	Q2B-1	Grains / m2 IR	2B	9.5	Par	Q2B-1
										Grains / m2 NI	2B	10.6	Par	Q2B-1
SW NI	2B	9.0	Gar	Q2B-2						SW NI	2B	6.1	Gar	Q2B-2
					YLD NI	2B	17.7	Gar	Q2B-2	YLD NI	2B	16.6	Gar	Q2B-2
					YLD IR	2B	17.0	Gar	Q2B-2	YLD IR	2B	13.9	Gar	Q2B-2
Grains / m2 NI	2D	14.9	Gar	Q2D-1										
SW NI	3A	6.1	Par	Q3A-1										
TGWT NI	3B	4.5	Gar	Q3B-1										
SW NI	4D	29.2	Par	Q4D-1	SW NI	4D	33.7	Par	Q4D-1	SW NI	4D	28.5	Par	Q4D-1
SW IR	4D	38.1	Par	Q4D-1	SW IR	4D	33.0	Par	Q4D-1	SW IR	4D	37.1	Par	Q4D-1
					TGWT NI	4D	16.6	Par	Q4D-1					
TGW IR	4D	5.2	Par	Q4D-1	TGW IR	4D	18.7	Par	Q4D-1	TGW IR	4D	11.1	Par	Q4D-1
					Grains / m2 NI	4D	25.4	Gar	Q4D-1	Grains / m2 NI	4D	12.7	Gar	Q4D-1
					Grains / m2 IR	4D	19.1	Gar	Q4D-1	Grains / m2 IR	4D	15.7	Gar	Q4D-1
Grains / m2 IR	5A	8.9	Gar	Q5A-1	Grains / m2 IR	5A	9.6	Gar	Q5A-1					
TGWT NI	5A	6.3	Gar	Q5A-2										
TGW IR	5A	6.5	Gar	Q5A-2										
SW IR	5A	6.8	Par	Q5A-2										
SW NI	5A	2.5	Gar	Q5A-3										
SW IR	5A	7.0	Gar	Q5A-3										
					TGWT NI	5A	5.0	Gar	Q5A-3					
					TGW IR	5A	7.1	Gar	Q5A-3					
					TGWT NI	5B	8.4	Gar	Q5B-1	TGWT NI	5B	10.7	Gar	Q5B-1
TGW IR	5B	14.2	Gar	Q5B-1	TGW IR	5B	9.2	Gar	Q5B-1	TGW IR	5B	14.6	Gar	Q5B-1
					Grains / m2 NI	5B	7.1	Par	Q5B-2					
										YLD IR	6A	8.9	Gar	Q6A-1
					TGWT NI	7A	12.3	Gar	Q7A-1	TGWT NI	7A	7.7	Gar	Q7A-1
					TGW IR	7A	8.2	Gar	Q7A-1					
Grains / m2 NI	7A	12.0	Par	Q7A-1	Grains / m2 NI	7A	7.8	Par	Q7A-1	Grains / m2 NI	7A	10.7	Par	Q7A-1
YLD NI	7B	16.8	Gar	Q7B-1										
					SW NI	7D	5.2	Gar	Q7D-1					

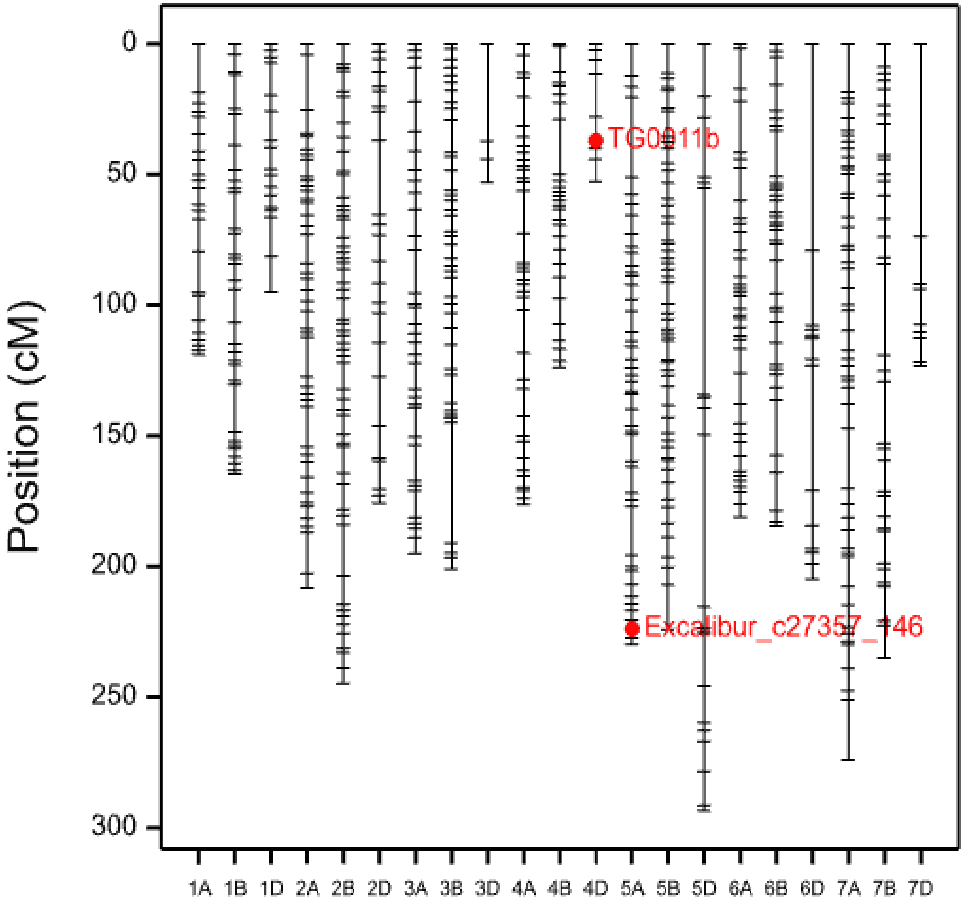
- Garcia beneficial alleles:
- 1A grain size
- 2B yield/SW QTL
- Rht-D1 grain number (reduced grain size)
- 5A grain size
- 5B grain size

Reduced canopy temp alleles correspond to large grain size

Mean of Not-Irrigated plots in 11.27 flight – 2 QTLs



QTL effects:
additive (blue=Parent 1 ; yellow-red=Parent 2)



Probably *Rht* = **Q4D-1**

= **Q5A-3**

Trait: %2018_TempNI
List of QTLs

Locus no.	Locus name	Linkage group	Position	-log10(P)
401	TG0011b	4D	37.06	4.402
454	Excalibur_c27357_146	5A	223.86	4.797

QTL effects

Locus no.	Locus name	%Expl. Var.	Add. eff.	High value allele	s.e.
401	TG0011b	10.018	0.337	Garcia	0.080
454	Excalibur_c27357_146	10.601	0.346	Paragon	0.078

Estimated lower and upper bounds of QTL positions

Locus no.	Locus name	Lower bound	Position	Upper bound
401	TG0011b	29.246	37.060	44.874
454	Excalibur_c27357_146	216.546	223.860	229.900

WGIN panel of parents for drought screening

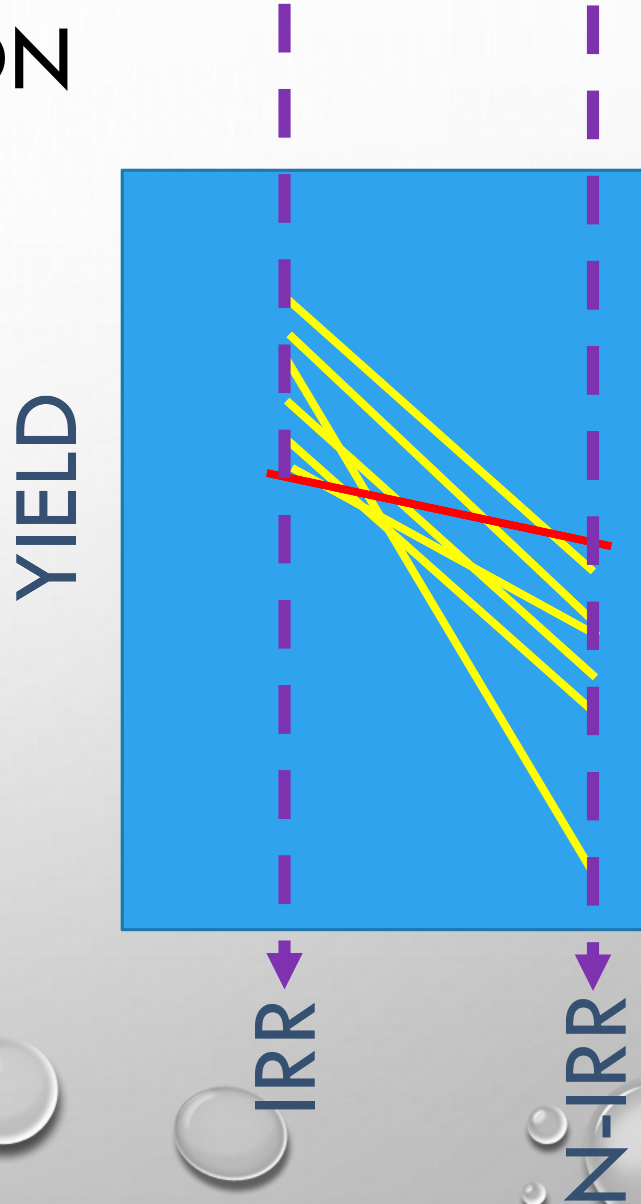
Possible Drought and/or Lodging lines	RL crosses	Parent of other crosses	CIMMYT	RothRes
Alchemy	■			
Atilla				
Avalon		■		■
Baj		■	■	
Barrel				■
Becard Kachu		■	■	
Beluga	■			
Borlaug 100		■	■	
Cadenza		■		■
Charger		■		
Chinese Spring				
CIMCOG 47		■	■	
CIMCOG 49		■	■	
Claire	■	■		■
Conqueror	■			
Cordiale	■			
Cordiale 3N (Rec 5-1)				
Costello				
Cougar	■			
Crusoe	■			■
Denman	■			
DFW BTK H17 (x3)				
DFW BTK H18 (x3)				
Einstein	■			
Fiorello		■		
Freiston				
Gallant	■			
Garcia		■		
Glasgow		■		
Grafton	■			
Graham	■			■
Hereward		■		■
Horatio	■			
Hylux				■
Icon	■			
Invicta	■			
Istabraq				■
JB Diego				
KWS Croft	■			
KWS Gator	■			
KWS Kielder	■			
KWS Santiago	■			
KWS Silverstone				
KWS Siskin				
KWS Sterling				
KWS Zyatt				

Possible Drought and/or Lodging lines	RL crosses	Parent of other crosses	CIMMYT	RothRes
LG Motown				
LG Skyscraper				
LG Sundance				
Lr19				
Malacca		■		■
Maris Widgeon				■
Mascani - OAT!				
MISR1		■	■	
Pamyati Azieva		■		
Panorama				
Paragon		■		■
Paragon EMS semi dwarves (x5)				
Paragon Rht D1 x B1				
Paragon RhtB1				
Paragon RhtB1 x Rht8				
Paragon RhtD1				
Paragon RhtD1 x Rht8				
Pastor				
Pfau		■	■	
Pomerelle		■		
Reflection				
Revelation	■			
RGT Illustrious				
Rht8				
Riband				■
Robigus		■		■
Savello				
Scout	■			
Siskin				■
Skyfall	■			
Soisson				■
Sokoll				
Solstice	■			■
Spark		■		
Super 152		■	■	
Synth Type		■	■	
Treasure		■		
Watkins 110				
Watkins Indian dwarfs W126		■		
Watkins Indian dwarfs W127		■		
Waxwing		■	■	
Weebill		■		
Wyalkatchem		■		
Xi19				■
Zyat				■
Paragon x Garcia RILs (15)				

- 1m^2 multiplication plots now
- Parents of biparental populations
- Chosen for drought and lodging.
- Also controls.

2019-2020 SEASON

- Replicated yield plots (6m^2) +/- irrigation
- Which parents show reduced yield penalty under drought?
- Repeat PxG approach or use subsets of RILs



Wgin Diversity Trial

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ROTHAMSTED
RESEARCH

Diversity 2019

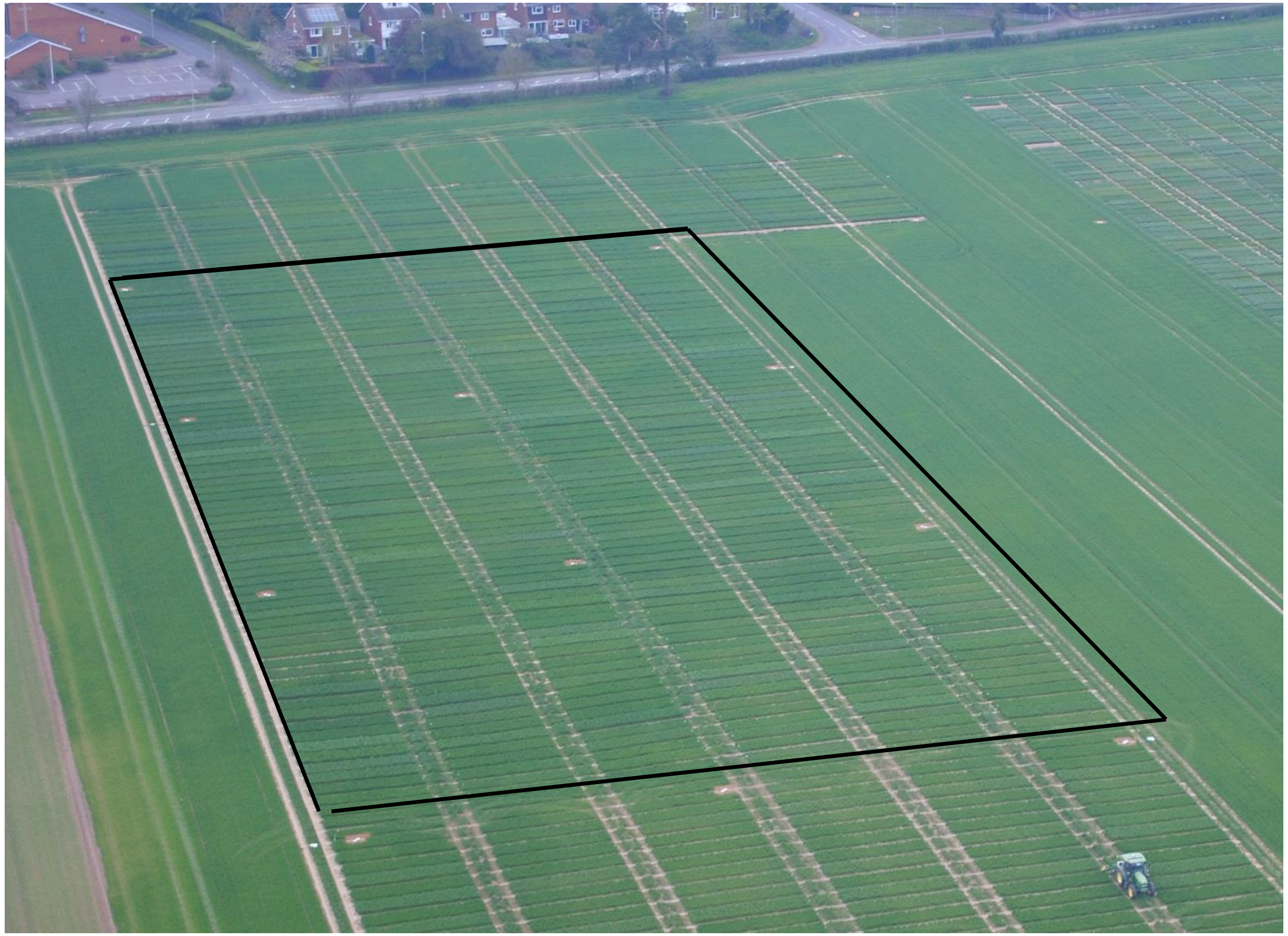
20 varieties
3 N levels (100, 200, 350)
Full and reduced fungicide/insecticide treatments
3 replicates

09/10/18	Sown
22/11/18	Sprayed with insecticide
28/02/18	Applied N(50kg/ha) + S
02/04/18	Applied T0 fungicide + PGR
03/04/18	Applied N (50, 100, 250)
02/05/18	Applied T1 fungicide

Images + hyperspectral reflectance collected on 10 occasions
15/2/19 – 03/05/19

ROTHAMSTED
RESEARCH

Diversity 2019



ROTHAMSTED
RESEARCH

2019 Varieties

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

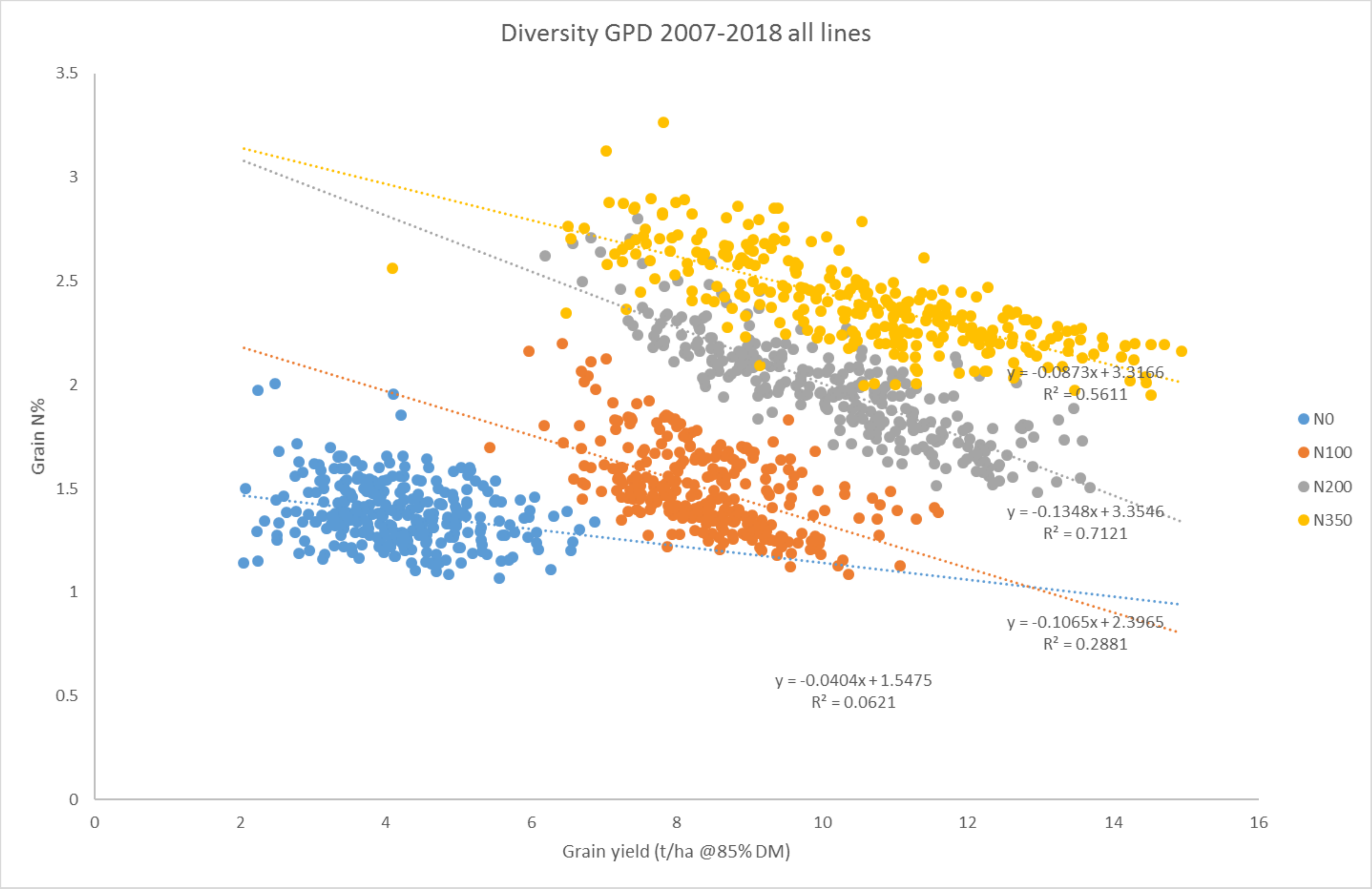
W=WGIN data, D=desk study

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

Variety	Source	Nabim	Rationale – can refer to previous years	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? Breadmaking?	MH	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	High yielding	MH, AR	18
10. KWS Zyatt	KWS	1	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

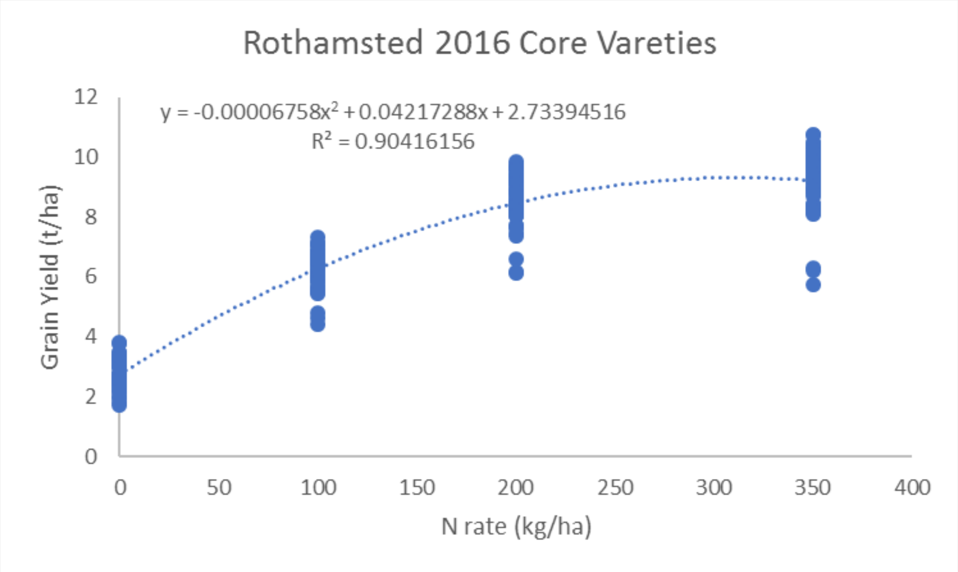
ROTHAMSTED RESEARCH

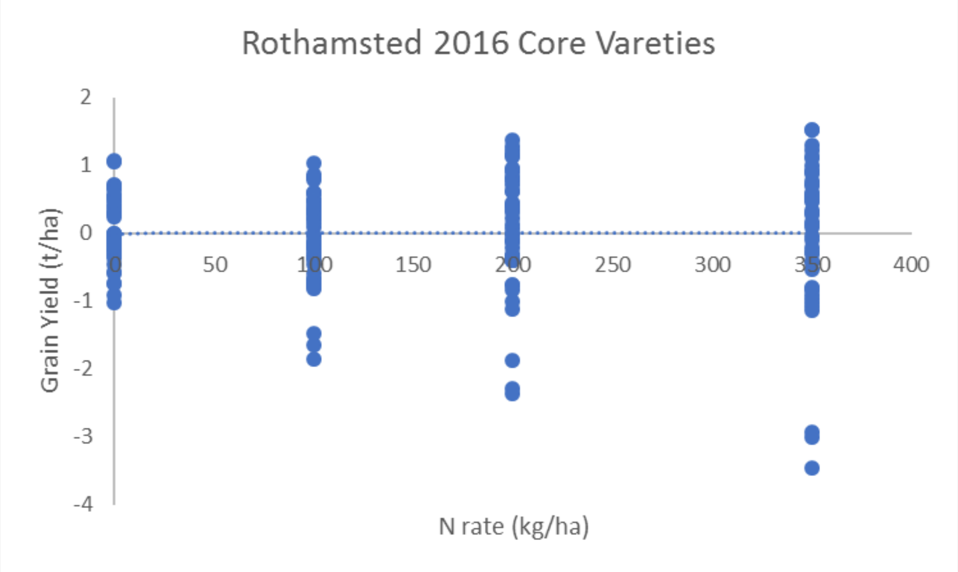
Grain Protein Deviation



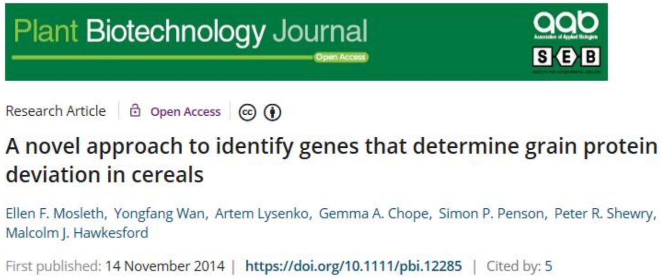
Diversity GPD 2007-2018 all lines

GPD – correcting for N fert & year effects


Rothamsted 2016 Core Vareties


Rothamsted 2016 Core Vareties

Step 1:
Correcting for fertilizer N effect on yield.
For each year (and site) grain yield plotted against N fertilizer.
For each plot the corrected yield is calculated as the actual yld – expected (ie the residual)


Plant Biotechnology Journal

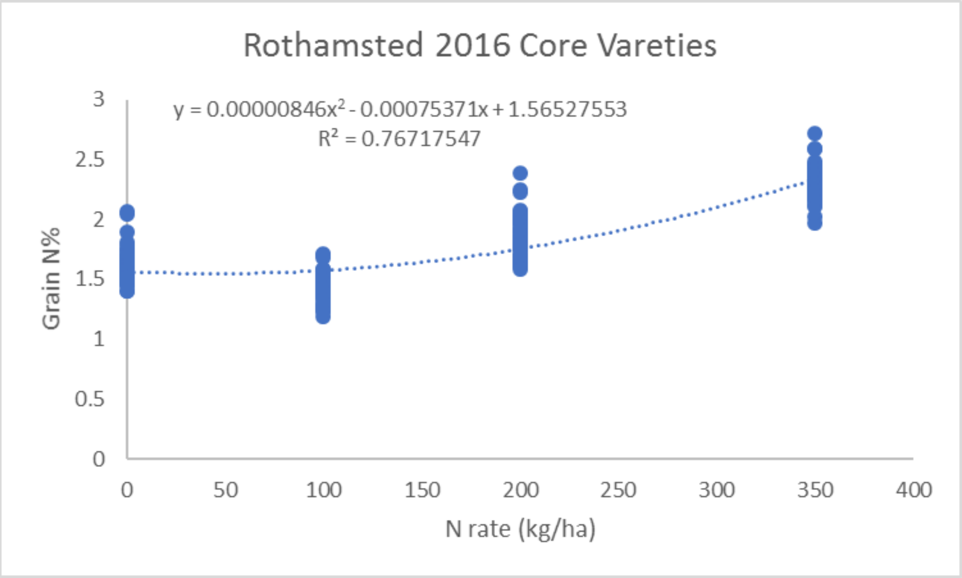
Research Article | Open Access

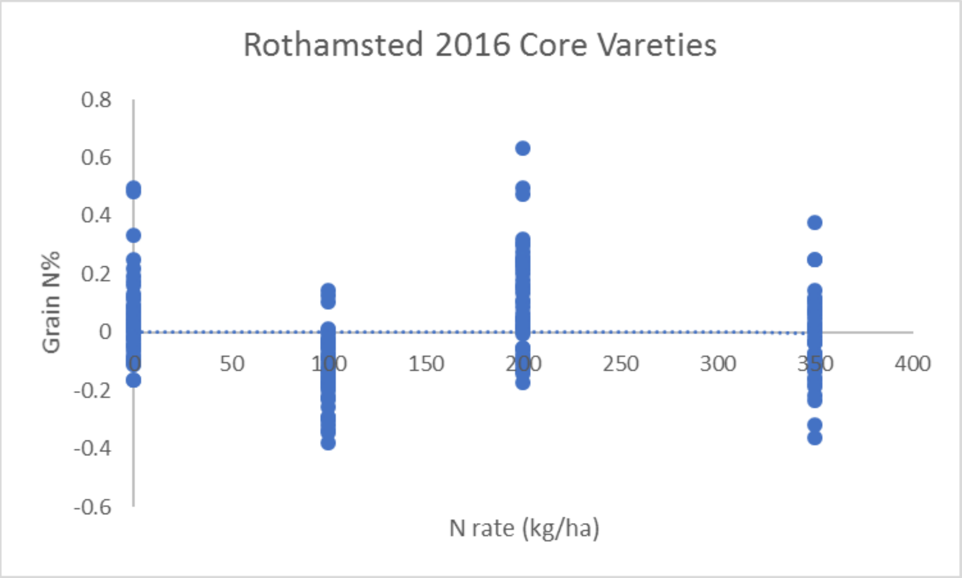
A novel approach to identify genes that determine grain protein deviation in cereals

Ellen F. Mosleth, Yongfang Wan, Artem Lysenko, Gemma A. Chope, Simon P. Penson, Peter R. Shewry, Malcolm J. Hawkesford

First published: 14 November 2014 | https://doi.org/10.1111/pbi.12285 | Cited by: 5

GPD – correcting for N fert & year effects





Step 2:
Correcting for fertilizer N effect on grain N.
For each year (and site) grain N plotted against N fertilizer.
For each plot the corrected grain N is calculated as the actual N% – expected N% (ie the residual)

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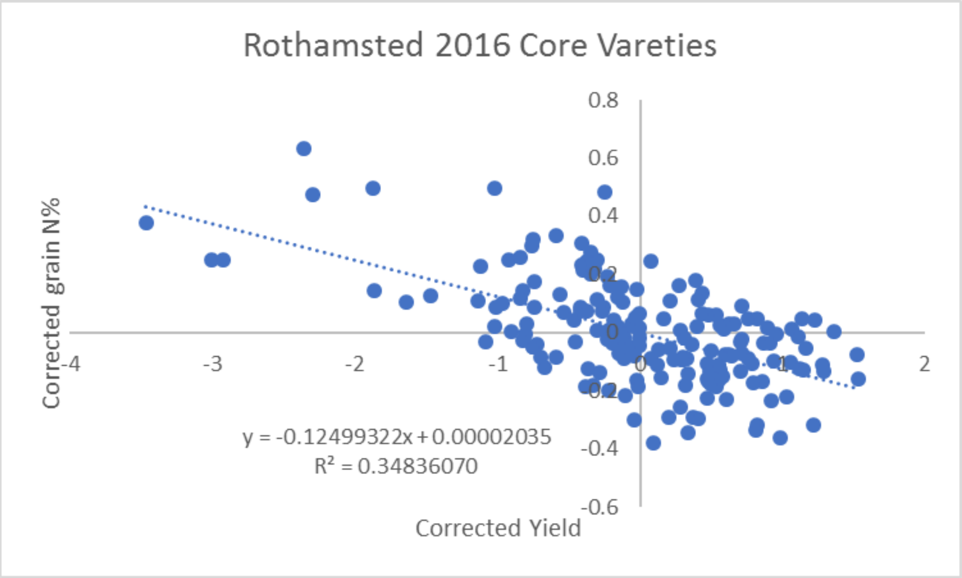
GPD – correcting for N fert & year effects



Step 3:
Calculating corrected GPD
The corrected grain N, plotted against corrected grain yield and. Residuals = corrected GPD

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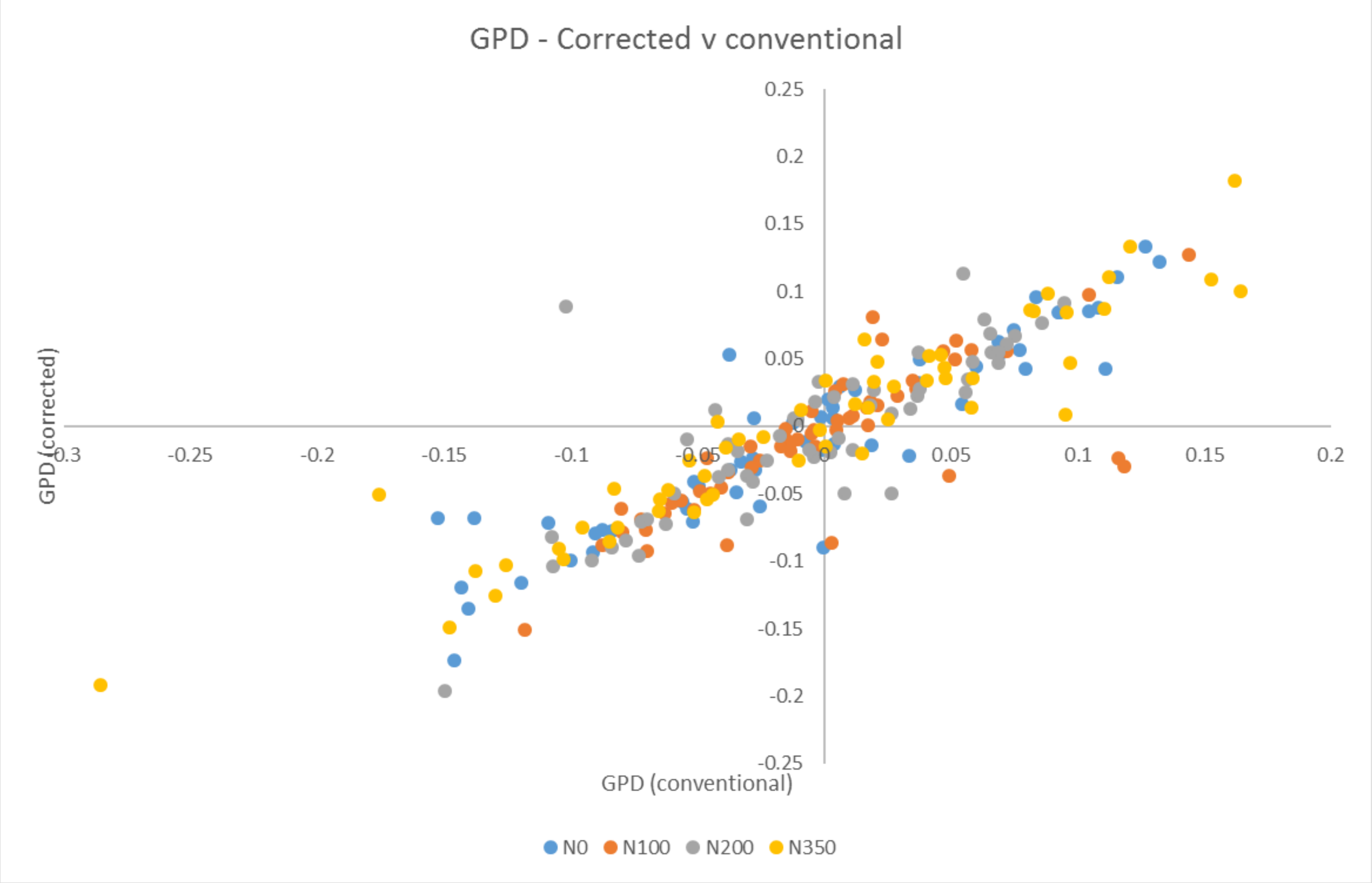
Effect of the corrections



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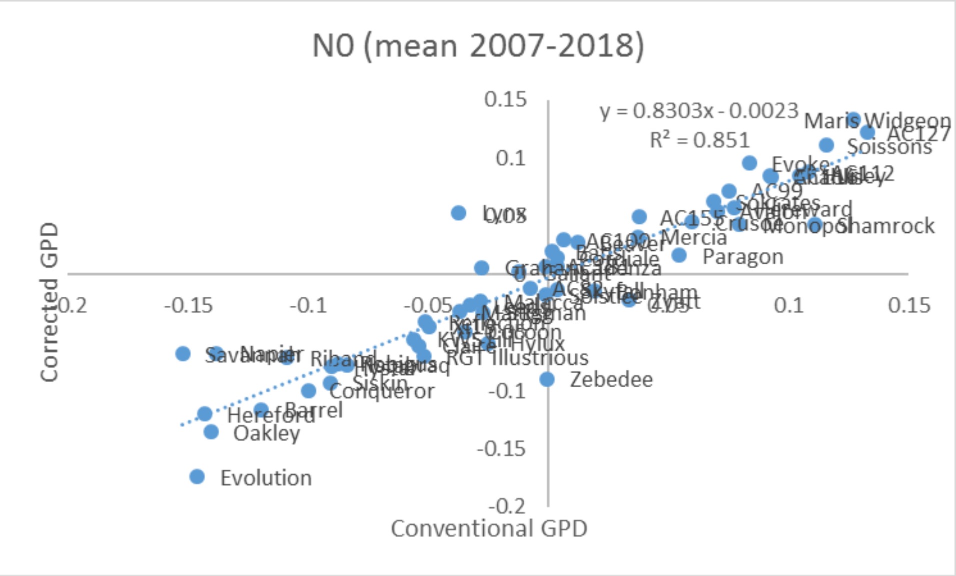
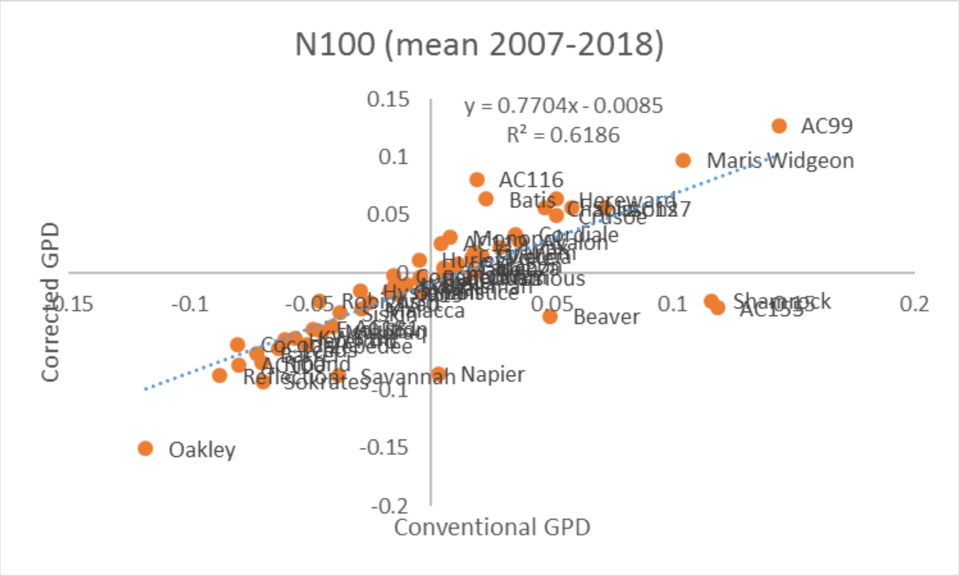
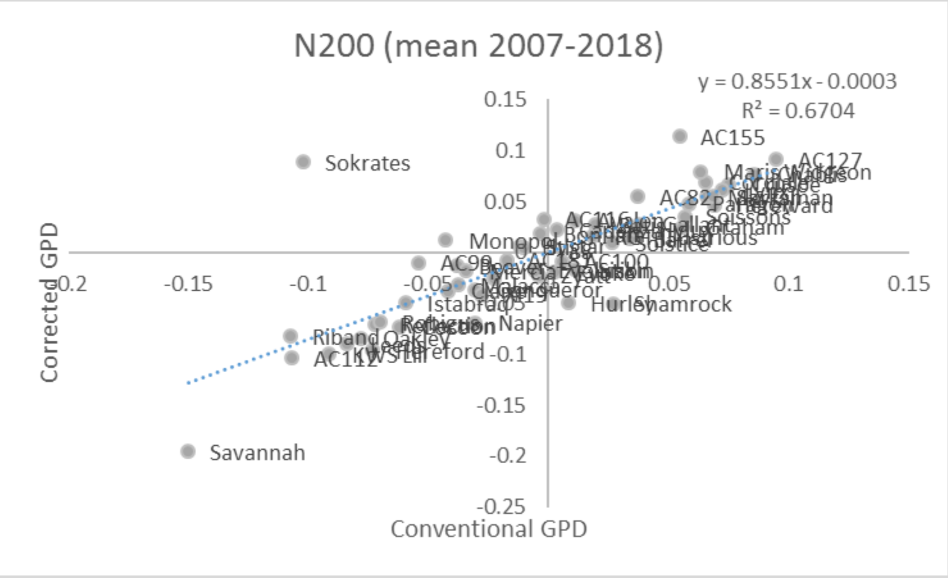
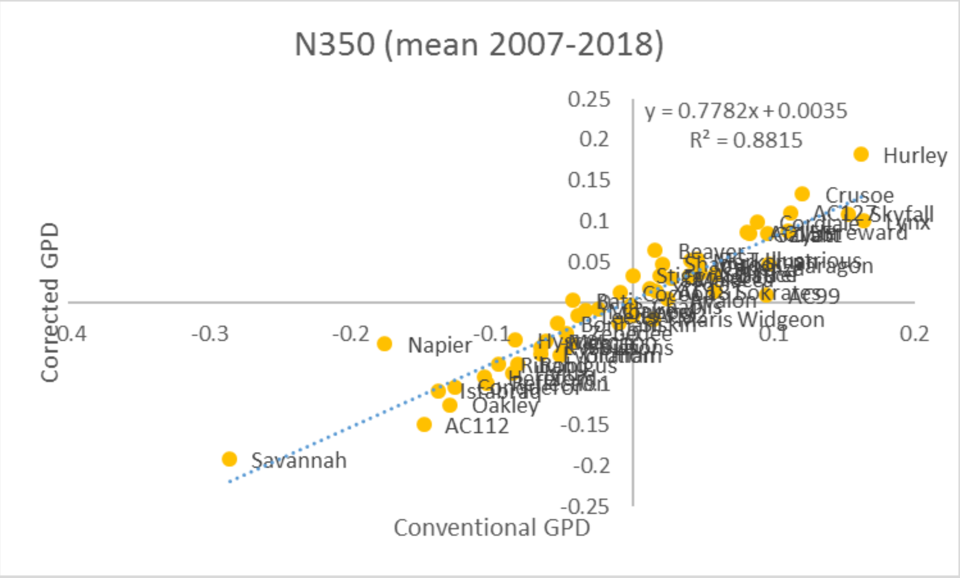
Effect of the corrections

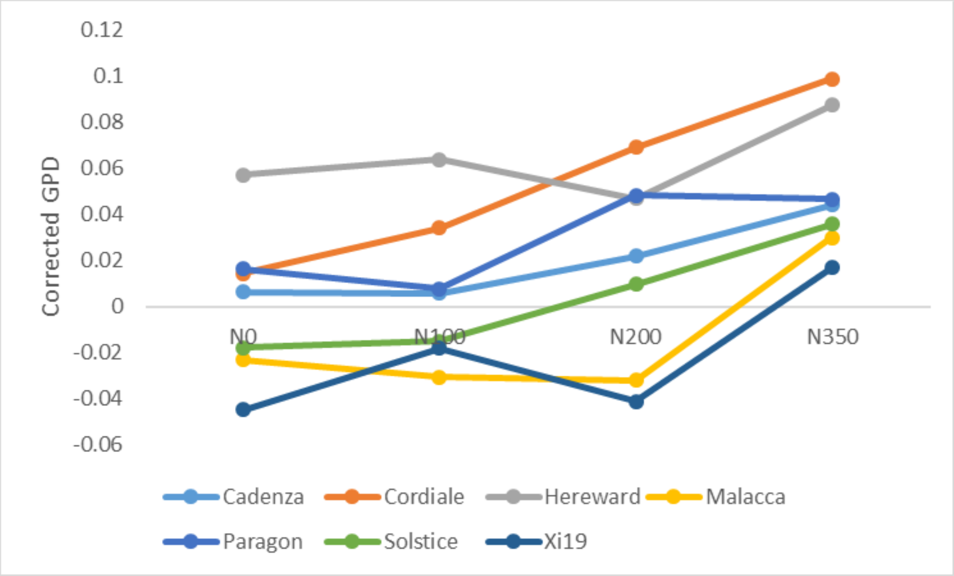


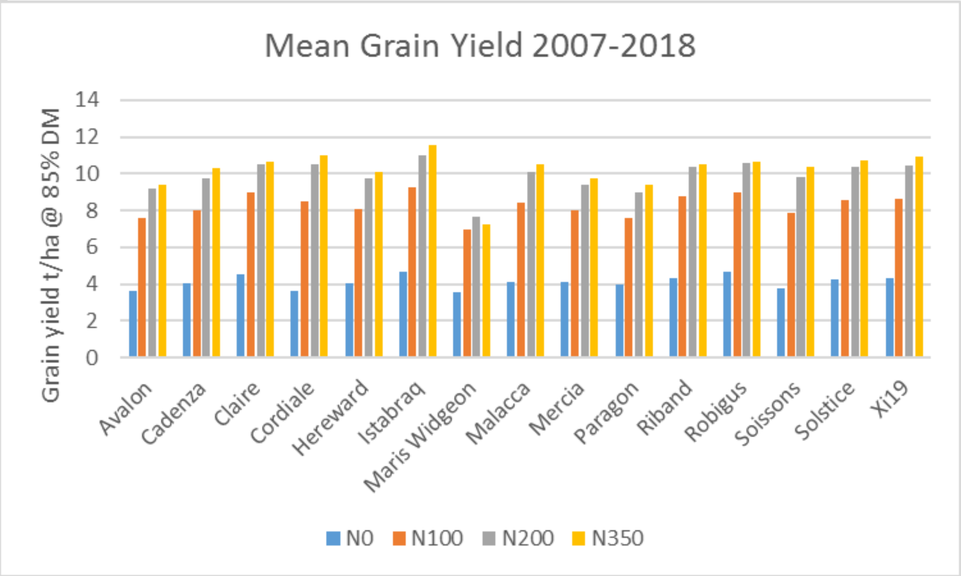


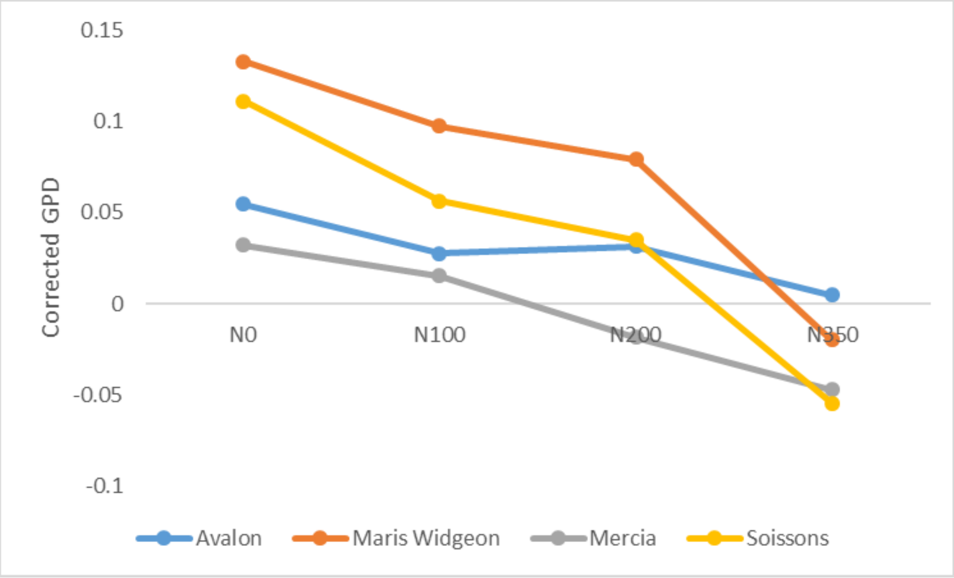


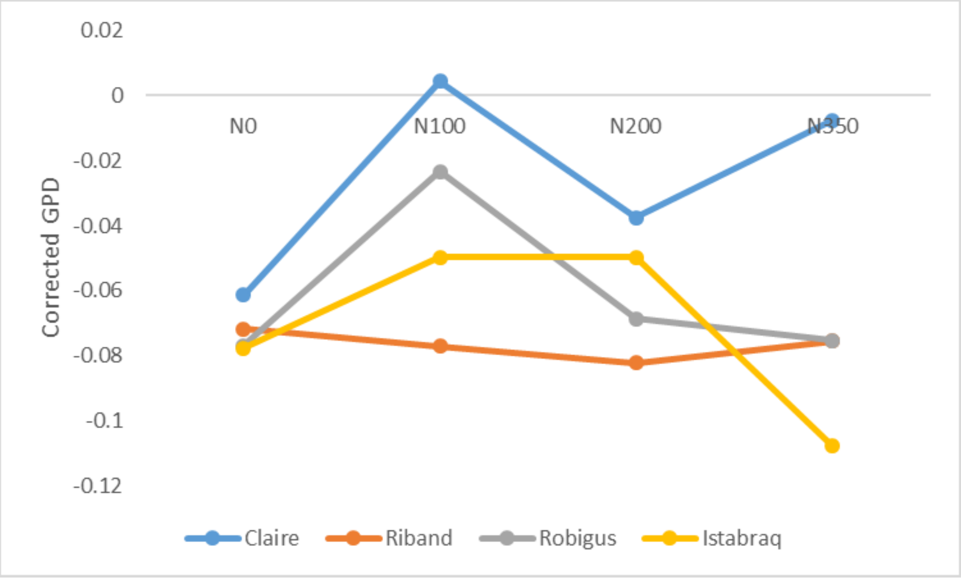


GPD – 15 core lines

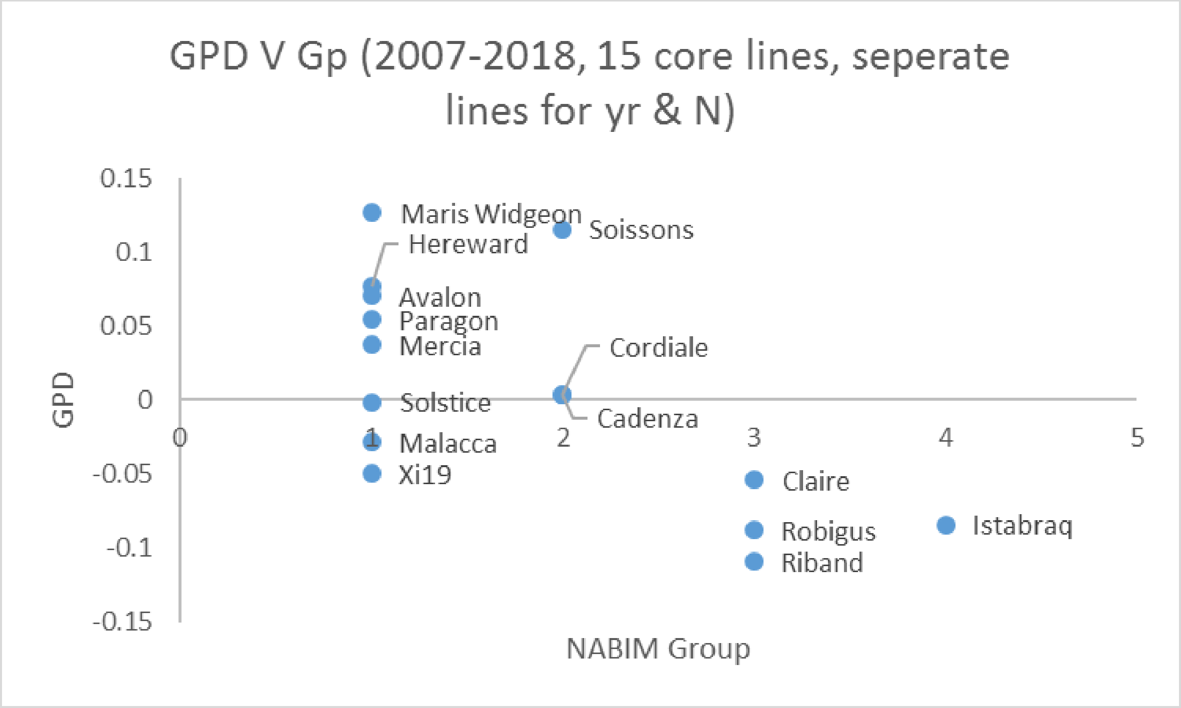



Mean Grain Yield 2007-2018





GPD & Group



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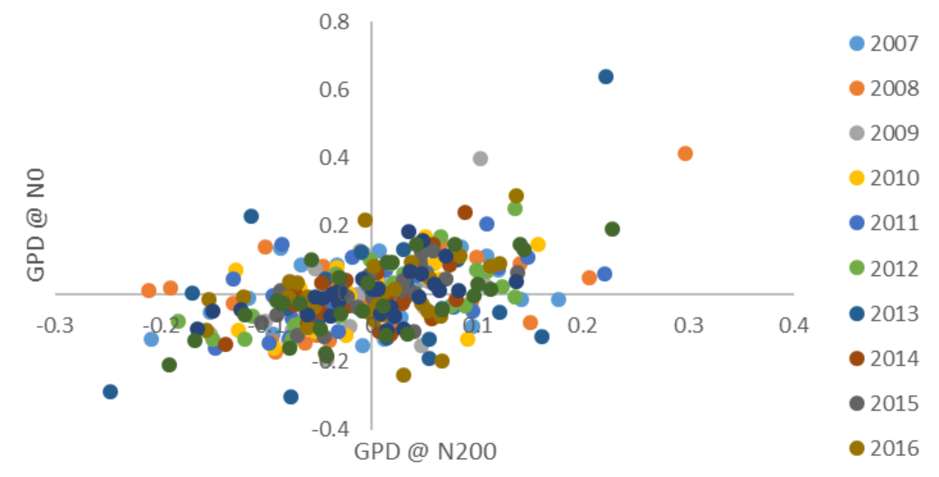
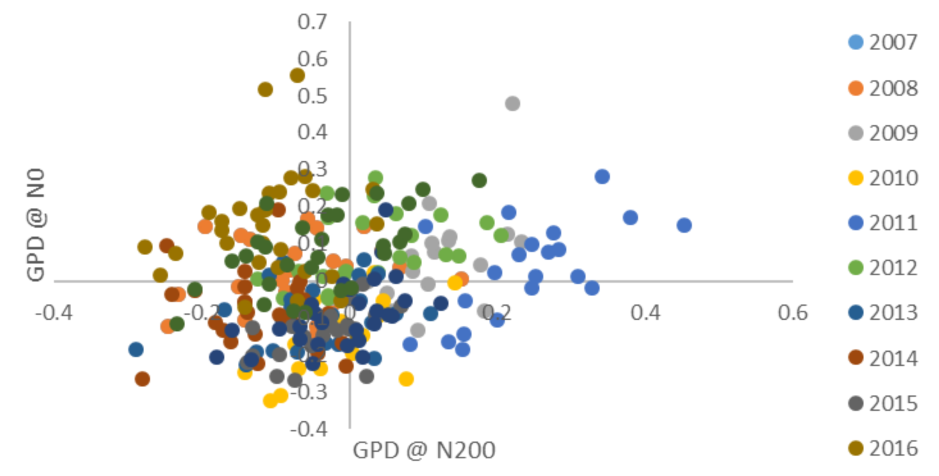
All lines included, means of reps

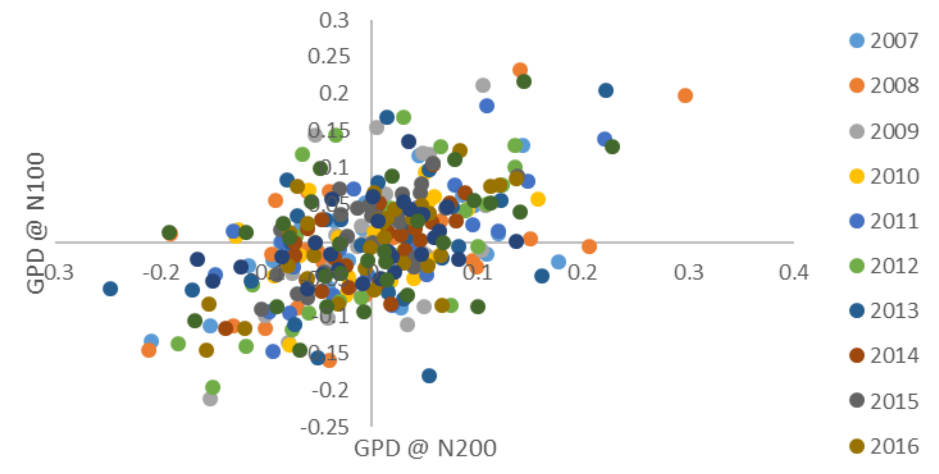
GPD 2007-2018, separate fit for each N rate by yr



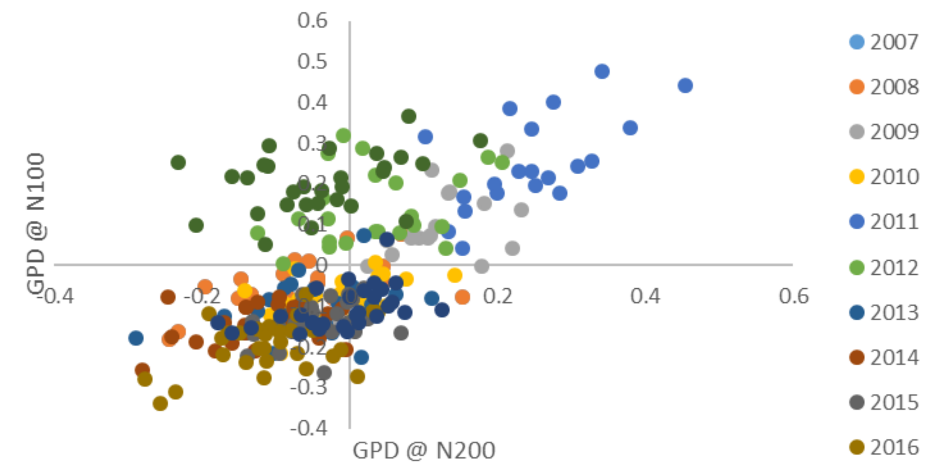
GPD - one fit over all yrs for each N level



GPD 2007-2018, separate fit for each N rate by yr



GPD - one fit over all yrs for each N level



Acknowledgments

Ellen Mosleth
Nicolas Virlet

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Rothamsted
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March Castle
David Steele

Farm Staff
Stephen Goward
Chris Mackay
Nick Chichester-Miles

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DFW

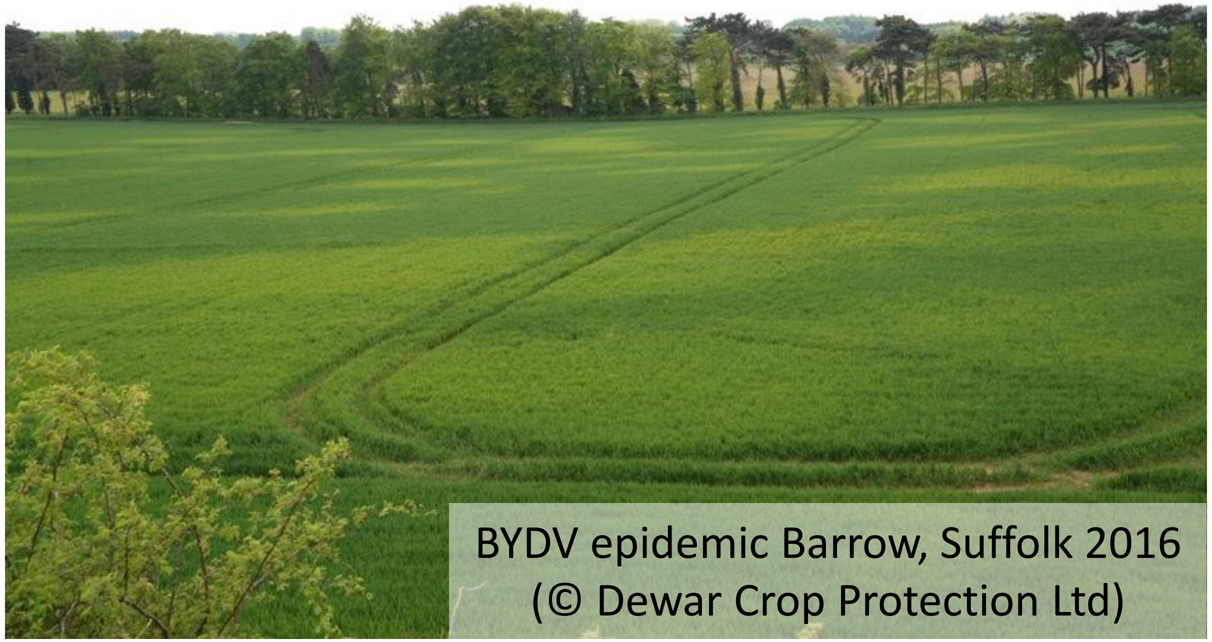
BYDV and wheat

Dr Gia Aradottir

Cereal aphids & Barley Yellow Dwarf Virus

Bird cherry-oat aphid

English grain aphid



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

WGIN Diversity trial - field

1	Avalon
2	Barrel
3	Cadenza
4	Claire
5	Crusoe
6	Graham
7	Hereward
8	Hylux
9	Istabraq
10	Malacca
11	Maris Widgeon
12	Mercia
13	Paragon
14	Robigus
15	Riband
16	Siskin
17	Soissons
18	Solstice
19	Xi19
20	Zyatt

Diversity trial – BYDV assessment

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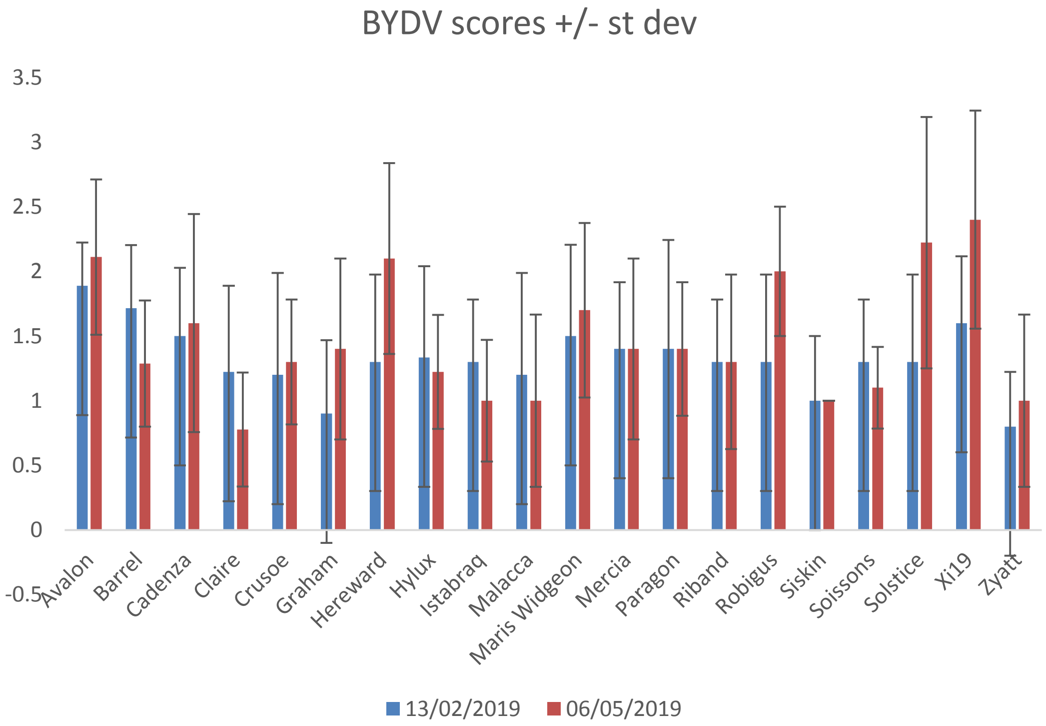

Next batch of lines tested

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1	W026
2	W115
3	W156
4	W170
5	W199
6	W208
7	W258
8	W374
9	W481
10	W546
11	W571
12	W579
13	W624
14	W639
15	W739
16	W742
17	W794
18	W821
19	Solstice
20	Paragon
21	Hereward

Next steps

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- Next 20 lines (selected from aphid phenotyping)
- Others if nominated
- Sample diversity trial for taqman assay
- Visit farmers for BYDV sampling (AHDB)

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Triticum monococcum Introgression

Michael Hammond-Kosack

WGIN MM May 8th 2019

@KWS

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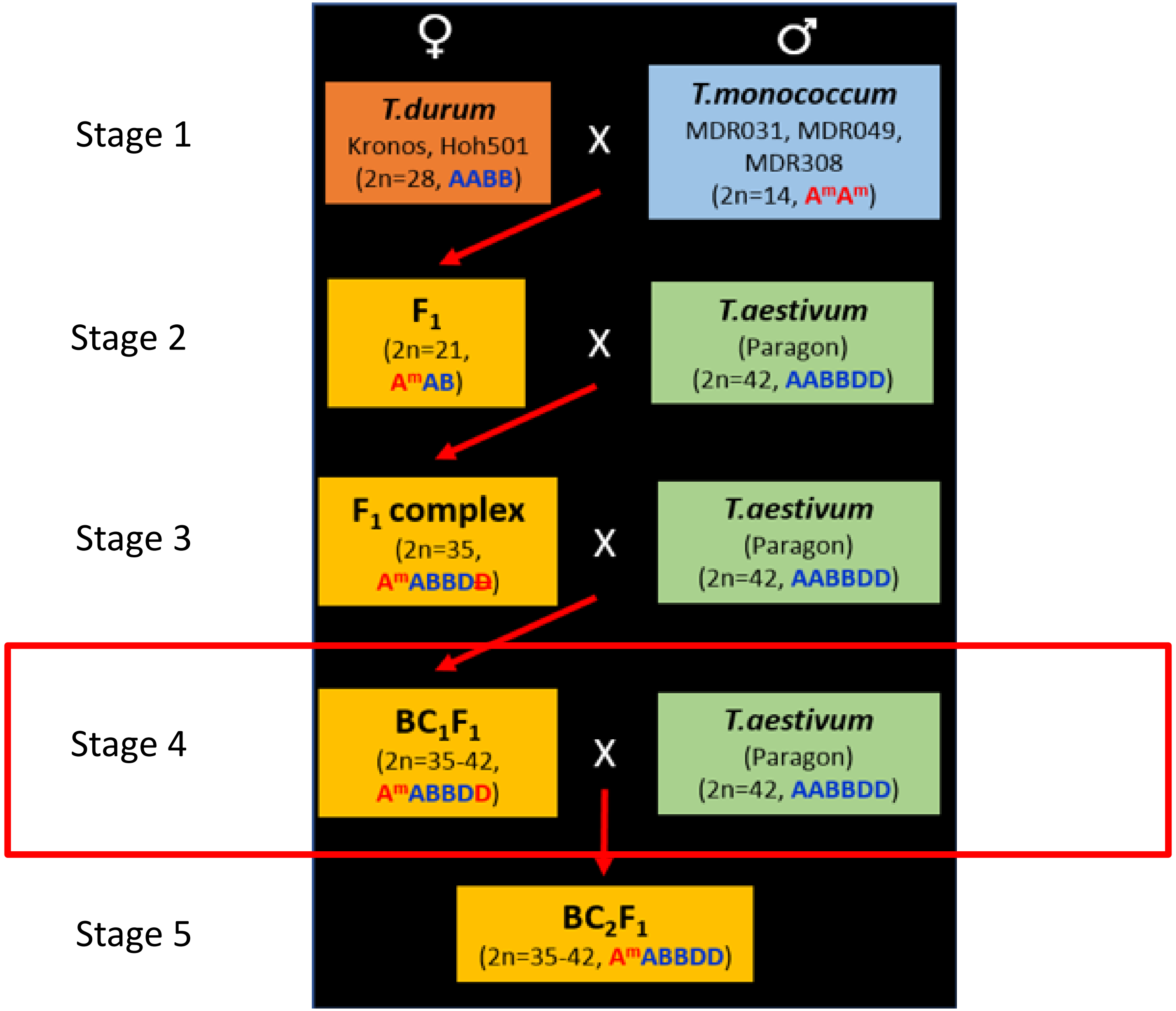

♀ ♂

Stage 1
T.durum Kronos, Hoh501 (2n=28, AABB) X **T.monococcum** MDR031, MDR049, MDR308 (2n=14, A^mA^m)

Stage 2
F_1 (2n=21, A^mAB) X **T.aestivum** (Paragon) (2n=42, AABBDD)

Stage 3
F_1 complex (2n=35, A^mABBD~~D~~) X **T.aestivum** (Paragon) (2n=42, AABBDD)

Stage 4
BC_1F_1 (2n=35-42, A^mABBDD) X **T.aestivum** (Paragon) (2n=42, AABBDD)

Stage 5
BC_2F_1 (2n=35-42, A^mABBDD)

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current state (May 7th)

		Plants in Glasshouse					
	plant	**BC1**	**Self**	**BC2 crosses (ears)**	**developing grains**	**Self crossed w Paragon (ears)**	**grains**
Kronos	F1C#24	5	0	9	**9**	n/a	
	F1C#47-1	2	1	8	too early	1	**12**
Hoh501	F1C#86-1	8	9	too early			
	F1C#86-2	2	0	too early			
	F1C#94	11	2	too early			
	round 2	**F1C**		**BC1 crosses**			
Hoh501	MDR049	1		too early			
	MDR308	5		too early			

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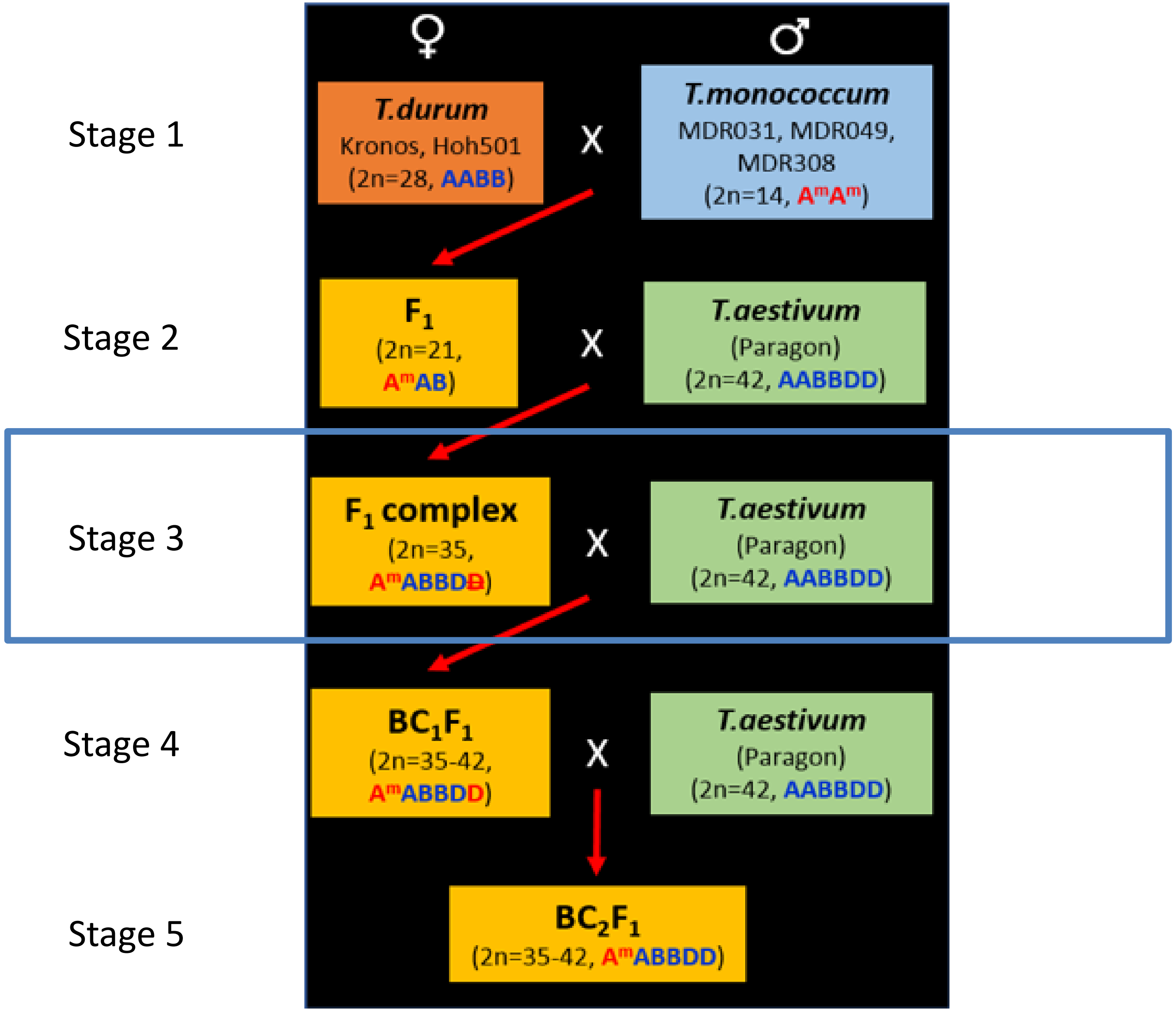

♀ ♂

Stage 1

T.durum
Kronos, Hoh501
(2n=28, AABB)

X

T.monococcum
MDR031, MDR049, MDR308
(2n=14, A^mA^m)

Stage 2

F_1
(2n=21, A^mAB)

X

T.aestivum
(Paragon)
(2n=42, AABBDD)

Stage 3

F_1 complex
(2n=35, A^mABBD~~D~~)

X

T.aestivum
(Paragon)
(2n=42, AABBDD)

Stage 4

BC_1F_1
(2n=35-42, A^mABBDD)

X

T.aestivum
(Paragon)
(2n=42, AABBDD)

Stage 5

BC_2F_1
(2n=35-42, A^mABBDD)

round 2 for
- aphid resistance
- septoria resistance

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current state (May 7th)

		Plants in Glasshouse					
	plant	**BC1**	**Self**	**BC2 crosses (ears)**	**developing grains**	**Self crossed w Paragon (ears)**	**grains**
Kronos	F1C#24	5	0	9	**9**	n/a	
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	F1C#94	11	2	too early			
	round 2	**F1C**		**BC1 crosses**			
Hoh501	MDR049	1		too early			
	MDR308	5		too early			

questions:

1. is it worthwhile/ sensible to cross the selfed F1Complex plants with Paragon?

2. can anything be gained from using Paragon **both** as male (as in crossing scheme) **and female** for the F1CBC1 plants?

Wheat genome exploitation through genomics analyses

Kostya Kanyuka
Mike & Kim Hammond-Kosack
Dan Smith

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Agricultural
Trust

Wall-associated receptor-like kinases (WAKs) are emerging as important new players in cereal disease resistance

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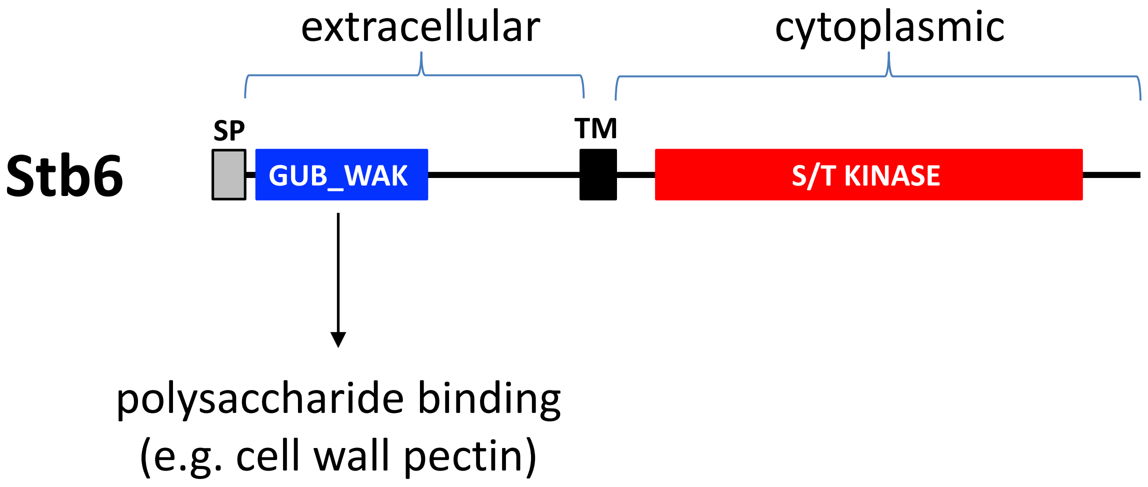
Stb6 is the first example of this class of extracellular receptors conferring gene-for-gene resistance to a pathogen



Other cloned WAK genes (wheat & other species):

WAK1	Arabidopsis	DAMP (OGs) receptor
RFO1	Arabidopsis	R gene (*Fusarium oxysporum*)
Htn1	maize	R gene (*Exserohilum turcicum*)
qHSR1	maize	R gene (*Sporisorium reilianum*)
Xa4	rice	R gene (*Xanthomonas oryzae*)
Snn1	wheat	S gene (*Parastagonospora nodorum*)

- qualitative pathogen resistance in a gene-for-gene manner (*Stb6*, *Xa4*)
- broad-spectrum, but partial, quantitative resistance (*Htn1*, *qHSR1, RFO1*)
- sensitivity to necrotrophic effector (*Snn1*)
- DAMP receptor recognising products of pectin degradation (*WAK1*)

Genome-wide sequence analysis of WAKs using exome and promotome capture

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Objectives:

1. Develop a myBaits array covering coding and promoter sequences of **all 601 WAK genes** identified in the wheat reference genome (cv. Chinese Spring)
2. Capture and sequence WAKs from 96 wheat lines with known responses to Septoria
3. Explore the association between known Septoria resistance loci and sequence polymorphism in specific WAK genes

arbor biosciences

an array containing 25,810 baits was designed and manufactured

Used to capture WAK sequences from 96 wheat genotypes

- 20 exotic wheats carrying known Septoria resistance genes
- 14 Watkins lines showing high level of resistance to multiple foliar diseases
- 60 wheat cultivars mostly of UK/ European origin with known field reaction to Septoria
- 2 *Triticum monococcum* genotypes with contrasting response to Septoria

Sequencing of captured DNA is in progress

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Genome-wide sequence analysis of WAKs using exome and promotome capture

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Status as of 7 May 2019:

- Illumina 150 nt reads have been trimmed for adaptor contamination and for quality at the 3’ end. Reads shortened to less than 40 nt were discarded. This produced more discards on the reverse reads (this is typical). The loss rate of reverse reads was <10% with **fewer than 2% of read pairs discarded**.
- Reads were retested for quality with FastQC and all issues were resolved
- Paired reads were mapped with BWA (v0.7.17) to the IWGSC release 43 reference (cv. Chinese Spring genome)
- The data is ready for sequence depth analysis and presence/absence determination, and SNP calling
- Determining copy number variation in exome capture data is less straightforward than for full shotgun data due to bait coverage and overlap, repeat content and GC variation. Dan has installed CNVkit (Talevich et al., 2016) which compensates for this for evaluation.

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Triticum monococcum chromosome 7Am sequencing and long-range assembly (towards isolation of *TmStb1*)

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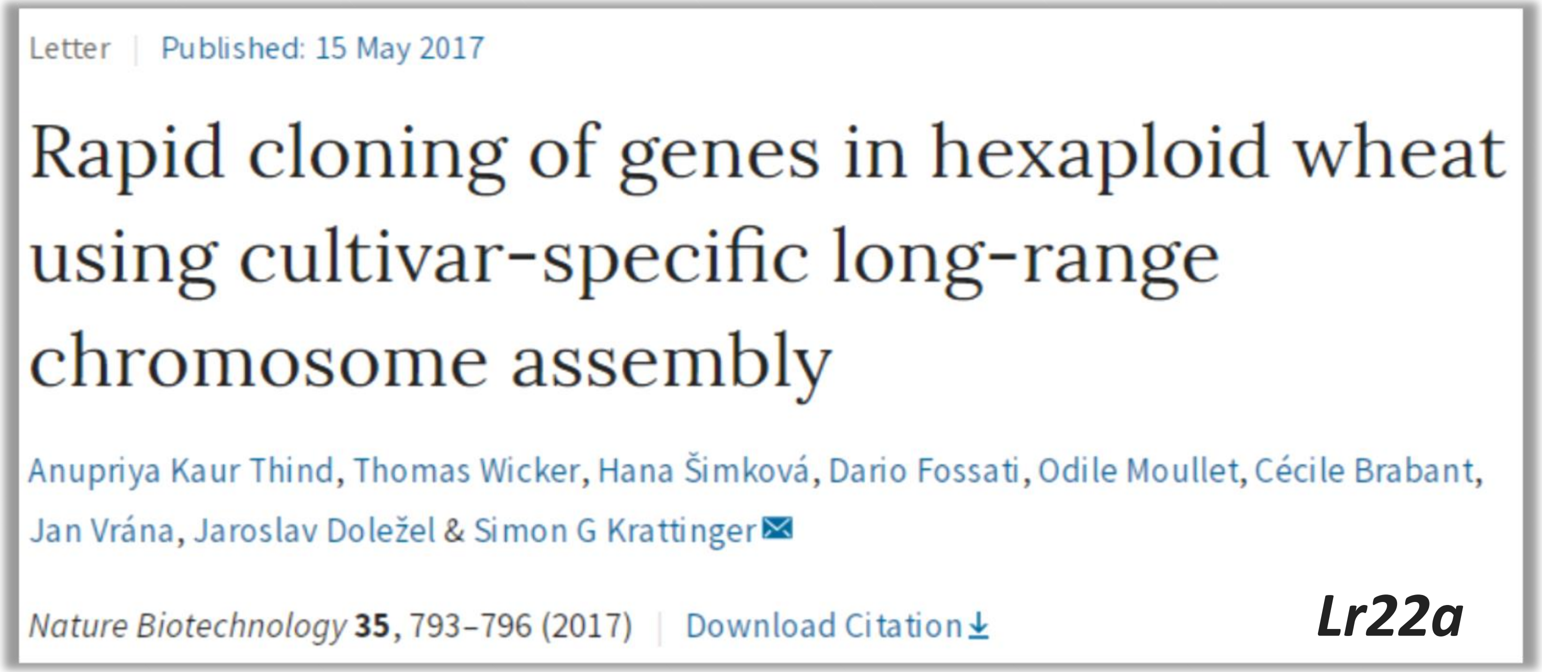

Letter | Published: 15 May 2017

Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly

Anupriya Kaur Thind, Thomas Wicker, Hana Šimková, Dario Fossati, Odile Moullet, Cécile Brabant, Jan Vrána, Jaroslav Doležel & Simon G Krattinger ✉

Nature Biotechnology **35**, 793–796 (2017) | Download Citation ⤓

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Triticum monococcum chromosome 7Am sequencing and long-range assembly

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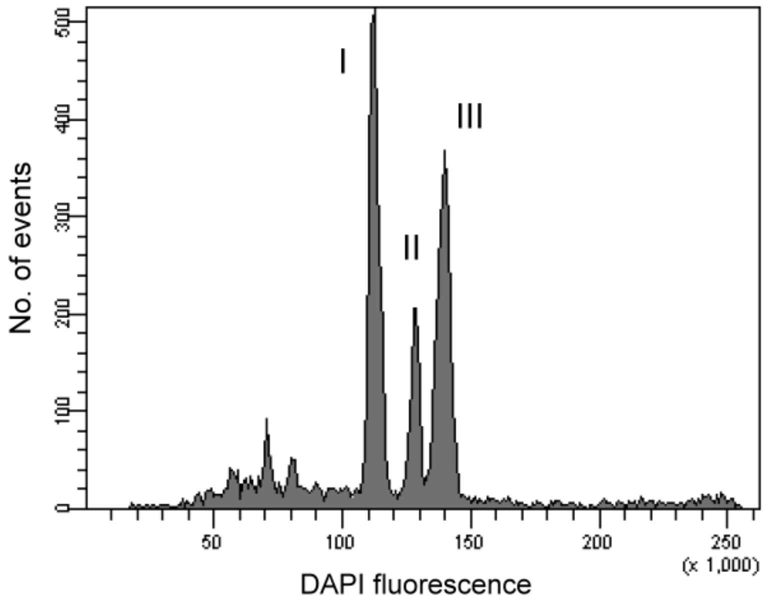
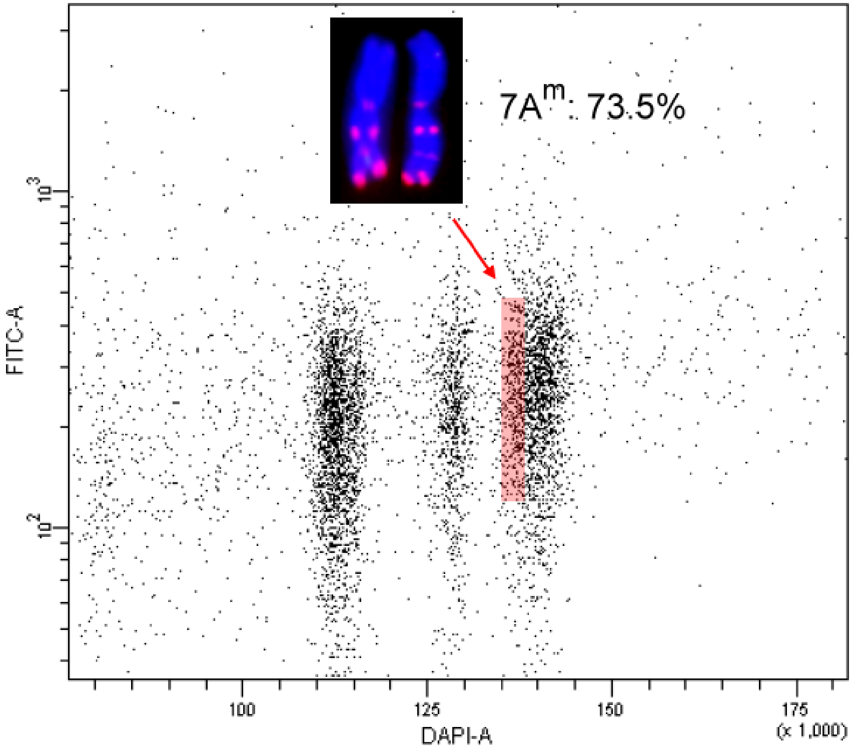
Objective 1:

- produce satisfactory pure HMW chr 7Am DNA in quantity (> 700 ng) sufficient for shotgun Illumina sequencing using flow sorting



Peak I: $1A^m$, $4A^m$, $6A^m$
Peak II: $5A^m$
Peak III: $7A^m$, $2A^m$, $3A^m$



Used FITC-conjugated GAA microsatellite repeats for FISHIS

collaboration with Institute of Experimental Botany CAS (Olomouc, Czech Republic)

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Triticum monococcum chromosome 7Am sequencing and long-range assembly

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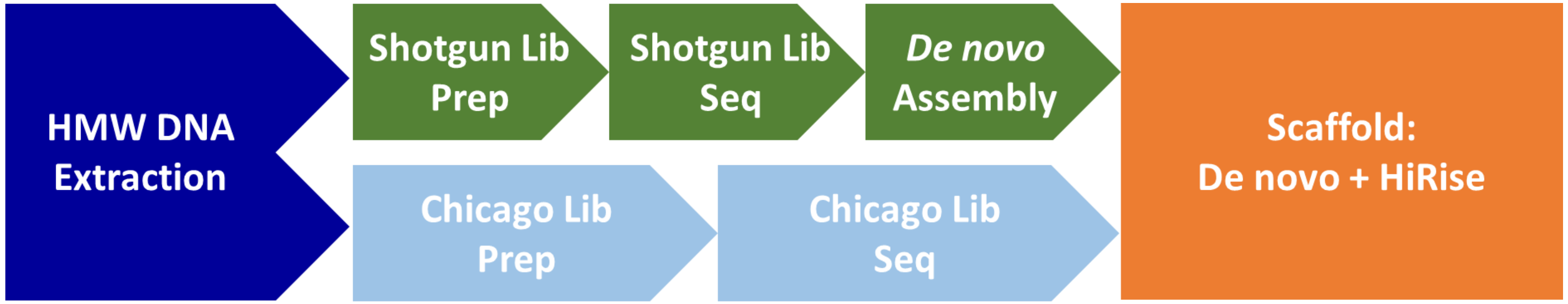
Objectives 2-3:

- shotgun sequence chr 7Am, and produce a draft *de novo* assembly
- generate scaffolded high-quality long-range assembly for chr 7Am using proximity ligation of an *in vitro*-reconstituted chromatin (Chicago)



collaboration with Dovetail Genomics (Santa Cruz, CA, USA)

Dovetail GENOMICS

Department for Environment Food & Rural Affairs

Wheat Genetic Improvement Network

Triticum monococcum chromosome 7Am sequencing and long-range assembly

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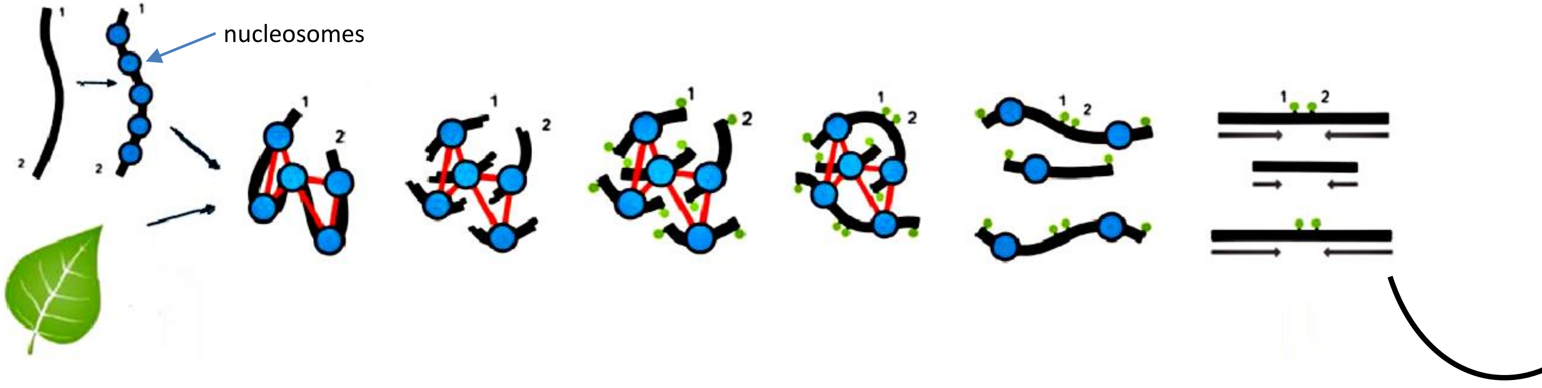
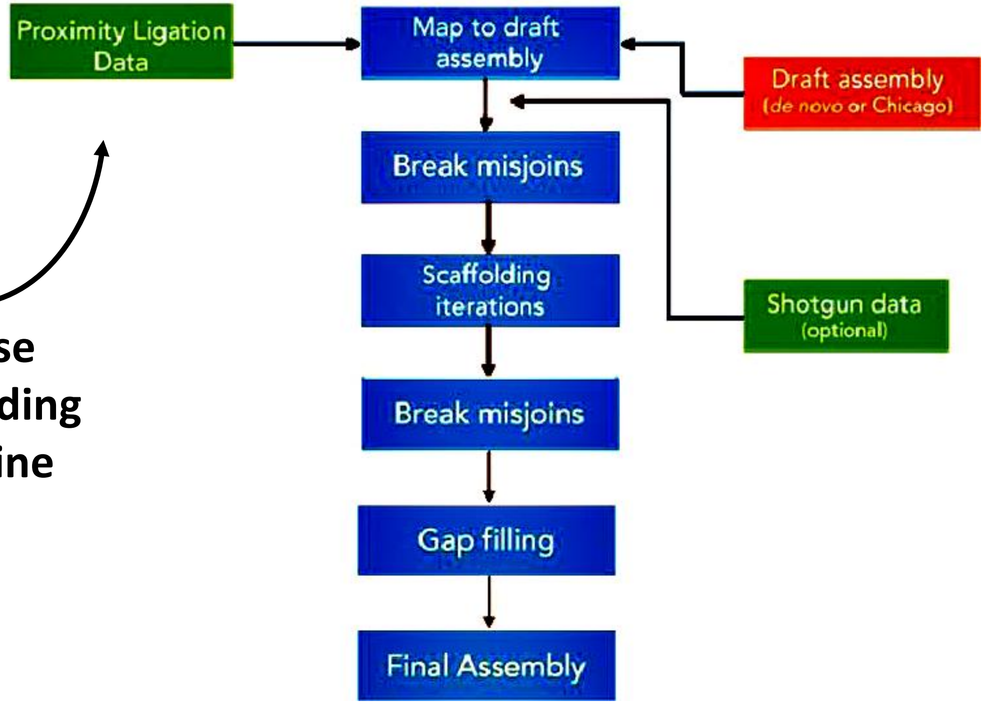


Chicago generates DNA libraries that are reconstituted into chromatin *in vitro*

HiRise scaffolding pipeline


Proximity Ligation Data
Map to draft assembly
Draft assembly (de novo or Chicago)
Break misjoins
Scaffolding iterations
Shotgun data (optional)
Break misjoins
Gap filling
Final Assembly

collaboration with Dovetail Genomics (Santa Cruz, CA, USA)

Dovetail GENOMICS

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