

Department for Environment Food & Rural Affairs



# Towards the Selection of RILs for Lodging, Anchorage and Drought Trials

*Clare Lister Simon Griffiths David Norris* 



Wheat

John Innes Centre

# **DALP** - <u>D</u>ROUGHT, <u>A</u>NCHORAGE and <u>L</u>ODGING <u>P</u>ANEL

CIMMYT lines and SATYN panel Elite varieties Roth Res and JIC lines DFW Breeders TK H17 and H18 EMS semi-dwarves Selected Paragon x Garcia RILs Watkins – all founder parents of NAM populations Other parents of available populations



- All (except one) founder parents of NAM populations present in DALP
- ~ 230 lines + / irrigation
- Spaced plants (~ 5.5. cm apart)
- 6m plots so able to irrigate with boom
- Usual field phenotyping (DTEM / HT / YLD)
- Lodging scores (after early June severe weather, will assess again)
- Will sacrifice specified segment of plots to anchorage tests

Assessing lodging traits from Paragon NAM background



- An additional year of the Paragon x Garcia Drought Trial was essential as only one spring drought occurred in the first three seasons
- Fortunately had strong spring drought in April 2019 so decision vindicated
- But this delayed scheduled exploration of new populations for lodging, anchorage and drought.
- Needed to compensate for this and explore more populations than originally planned
- Decided on mixed trial of a number of RIL populations the results of which should allow us a number of potential suitable populations to drill in autumn 2020

Assessing lodging traits from Paragon NAM background





- WGIN and DFW have 80+ populations of crosses between Paragon and founder parents (including the Watkins collection)
- Minimum of 96 RILs per founder parent
- Find marker associated traits for lodging with high accuracy
- Need to select 1-3 populations for autumn 2020 trials

**RIL Selection – 3-step process – Paragon is benchmark** 



- Selected RILs with phenotypes similar to Paragon, where possible
- 1) DTEM very similar heading date



2) Height

## 3) Yield

## From each population up to 11 lines were selected



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1) DTEM

## 2) Height – similar or slightly smaller



3) Yield

From each population up to 11 lines were selected



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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 – similar or slightly greater

## **Trial design and measurements**



- 466 RILs selected from 52 crosses (up to 11 lines / population)
- Drilled in 1 m plots, randomized design, single rep
- Phenotyping:
  - DTEM, HT, YLD
  - Lodging (after early June severe weather, will assess again)
  - Stiffness (currently concentrating on populations where some RILs lodged and some not)

## **Scoring Lodging and Stem Stiffness**



- Lodging scored 2-3 times over 10 days in all lines (NAM and DALP) after severe weather in early June
- Trying to identify a population(s) where lodging is a segregating trait
- Looked at NAM populations where some RILs had lodged but not many 13+ currently identified
- Some completely lodged, some no lodging
- Variation in severity of lodging, some lines recovered, others didn't
- Stem stiffness compared to Paragon

## Latest Lodging and Stem Stiffness Results

-	Wheat
	Genetic
Y	Improvement
B	Network

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	DA	RILs				
	DTEM from 1st May	Parent	Lodging	Stiffness (partial data)		
RIL popn				1 ( <p)< th=""><th>2 (=P)</th><th>3 (&gt;P)</th></p)<>	2 (=P)	3 (>P)
Paragon x Bacard Kachu	9.5	1	3 of 11	2	2	1
Paragon x Fiorello	21	x	3 of 11		4	
Paragon x Super 152	11.5	1	2 of 11	1	4	
Paragon x Waxwing	9	x	2 of 7	2		1
Paragon x Watkins 94	31	2	7 of 9		2	2
Paragon x Watkins 126	17	2	4 of 11	2	3	3
Paragon x Watkins 199	19.5	1	4 of 9	1	2	
Paragon x Watkins 224	28	3	5 of 10		1	4
Paragon x Watkins 471	24.5	3	4 of 11			6
Paragon x Watkins 496	25.5	x	3 of 11	1	2	2
Paragon x Watkins 560	26	3	5 of 10	1		3
Paragon x Watkins 694	21	2	6 of 11		1	4
Paragon x Watkins 749	26	3	3 of 10			4
Paragon	33.5	x				
		x - no lodging				
		1 - slight lodging				
		2 - medium lodging				

3 - severe lodging

## Selection of RIL population(s)



- Want to identify as many potential populations as possible having a range of 'lodgability' and stem stiffness
- Will need to do germination tests on these to check good level of viability
- Will select 1-3 population to drill this autumn +/- irrigation



# Diversity 2020





### Sown end Oct

Rather thin – not expecting good yields

Hyperspectral reflectance & UAV flights weekly from end-May

Disease assessments: 14-16 May 27-29 May 15 June 02 July











### For each experiment:

Operation	Hr	Notes
Planning flight	1	
Setting out GCPs & logging position, remove end of season	4 (incl two people)	Usually 2 people
Clear round GCPs	1	
Drawing shape file	2-8	

#### For each flight:

Operation	Time	
Flying time	1	Including charging batteries
Assigning GCPs & running software	0.5 * 2 for RGB + NIR	

Plus 18-32hrs computer time per image set (600 images) x2 for RGB and NIR







2018 Plant height meaned across varieties







2017 Plant height N200 SFP









**—**2019 **—**2018 **—**2017 **—**2016







**—**2019 **—**2018 **—**2017 **—**2016













**—**2019 **—**2018 **—**2017 **—**2016











#### Ear counts

Acknowledgments: Malcolm Hawkesford March Castle Fenner Holman Chris Mackay Deborah Wright





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# Triticum monococcum Introgression

(update 563.4)

virtual WGIN MM July 8th 2020

Michael Hammond-Kosack (RRes)



#### fertility for BC3 crosses for MDR031 derived plants



#### BC3 crossing fertility for F1C24 descendants



#### BC3 crossing fertility for F1C47-1 descendants







Hoh501





BC3 crossing fertility for F1C86-2 descendants



## Summary of BC3 yields for MDR031





Hoh501

BC3 grains obtained from each F1C86-2-BC2 plant 60 181 gr 40 20 BC2-X146-1 BC2-X146-2 BC2-X146-4 BC2-X146-5 BC2-X55-1







## Summary of BC2 fertilities and yields for MDR049 and MDR308

#### MDR049



BC2 grains obtained from each F1C[MDR049]-BC1 plant

BC2 crossing fertility for F1C[MDR308] descendants



BC2 grains obtained from each F1C[MDR308]-BC1 plant



#### MDR308

#### BC3 (BC2) grains for each F1C parent



T.mon MDR031 3



MDR308

Kronos 9



diploid

MDR049

tetraploid

hexaploid































### selfed BC1 ears from MDR049 & MDR308 derived plants















## T. monococcum introgression field trial April 2020 onwards

## **Purpose :** 1. To assess various traits as a 1<sup>st</sup> wheat crop

- Plant growth
- Plant development
- Responses to Yellow Rust and Septoria
- Ear morphology and grain shape
- 2. Seed multiplication for Oct 2020 Take-all trial (3<sup>rd</sup> wheat)

## WGIN's 1st Triticum monococcum Introgression Field Trial

Design #	ALL SELFS (S-#)	F1hybrid		F1C	BC1	BC2	Design #		
1	BC1-X12-1			Fielder			33-36	Paragon	
2	BC1-X12-3						37	Fielder	
3	BC1-X87-1	Kronos					38	Cadenza	
4	BC1-X173-1	KIOHOS					39	Kronos	Take-all R Aphid R Septoria R
5	BC1-X27-1						40	Hoh501	
6	BC1-X147-1						30	MDR031	
7	BC1-X75-1						31	MDR049	
8	BC1-X88-1						32	MDR308	
9	BC1-X122-1								
10	BC1-X161-3		MDR031						
11	BC1-X186-2								
12	BC1-X187-1								
13	BC1-X187-2								
14	BC1-X187-3								
15	BC1-X74-1				Paragon	Paragon			
16	BC1-X115-1			Paragon					
17	BC1-X189-2								
18	BC1-X224-1	Hoh501							
19	BC1-X224-2								
20	R2#2-7		MDR049						
21	R2#13-1-1		MDR308						
22	R2#14-1-1		1010300						
23	R2#16-7			31					
24	BC2-X65-1								
25	BC2-X113-1		MDR031						
26	BC2-X113-3								
27	BC2-X150-1								
28	BC2-X150-2								
29	BC2-X150-3								
Trial Design and Layout







Late April 1. Lockdown pre-germination @ home

#### Early to late May

2. Nursery (RRes glass house)





## 25<sup>th</sup> May 2020

3A Trial site preparation (3 weeks after glyphosate treatment ) and then cultivated 28<sup>th</sup> May



#### 29<sup>th</sup> May 2020

3. trial lay-out



4. the first row planted



Water bowser

#### 5. getting there



6. All done...



7. ...apart from watering twice a day (2 x 100l)



3,500 litres so far, plus the occasional rain

**Disease** development - Septoria – none Yellow rust – a lot

#### **Basal leaves heavily YR infected**

1 control + 4 lines

40. Hoh501
2. BC1 – X12-3
6. BC1 - X147-1
8. BC1 – X88-1
29. BC2 – X150-3

3 Tm lines – no yellow rust lesions

Design #	ALL SELFS (S-#)	F1hy	/brid	F1C	BC1	BC2	
1	BC1-X12-1			Fielder	-		
2	BC1-X12-3			Fielder			
3	BC1-X87-1	Kronos					
4	BC1-X173-1	KIONOS					
5	BC1-X27-1						
6	BC1-X147-1						
7	BC1-X75-1						
8	BC1-X88-1						
9	BC1-X122-1						
10	BC1-X161-3		MDR031				
11	BC1-X186-2						
12	BC1-X187-1						
13	BC1-X187-2						
14	BC1-X187-3						
15	BC1-X74-1				Paragon	Paragon	
16	BC1-X115-1			Paragon			
17	BC1-X189-2						
18	BC1-X224-1	Hoh501					
19	BC1-X224-2						
20	R2#2-7		MDR049	]			
21	R2#13-1-1		MDB308				
22	R2#14-1-1		IVIDASUO				
23	R2#16-7			]			
24	BC2-X65-1						
25	BC2-X113-1						
26	BC2-X113-3		MDR031				
27	BC2-X150-1						
28	BC2-X150-2						
29	BC2-X150-3						

### Plant development – as of 7<sup>th</sup> July 2020

Most plants in anthesis (GS60 onwards)

3 controls and 5 lines

30. MDR031
33. Paragon
39. Kronos
1. BC1 – X12-1
6. BC1 – X147-1
8. BC1 – X88-1
17. BC1 – X189-2
27. BC2- X150-1

Also have high YR in basal leaves

	Design #	ALL SELFS (S-#)	F1hy	brid	F1C	BC1	BC2	
	1	BC1-X12-1			Fielder			
	2	BC1-X12-3			Fielder			
	3	BC1-X87-1	Kronos					
	4	BC1-X173-1	KIONOS					
	5	BC1-X27-1						
	6	BC1-X147-1						
	7	BC1-X75-1						
	8	BC1-X88-1						
	9	BC1-X122-1						
	10	BC1-X161-3		MDR031				
	11	BC1-X186-2						
	12	BC1-X187-1						
	13	BC1-X187-2						
	14	BC1-X187-3						
	15	BC1-X74-1				Paragon	Paragon	
	16	BC1-X115-1			Paragon			
	17	BC1-X189-2						
	18	BC1-X224-1	Hoh501					
	19	BC1-X224-2			_			
	20	R2#2-7		MDR049				
	21	R2#13-1-1		MDR308				
	22	R2#14-1-1		11011300				
	23	R2#16-7						
	24	BC2-X65-1						
	25	BC2-X113-1						
	26	BC2-X113-3		MDR031				
	27	BC2-X150-1						
	28	BC2-X150-2						
۲	29	BC2-X150-3						

### Plant development – as of 7<sup>th</sup> July 2020

Most plants only up to GS 37 (i.e. flag leaf visible)

1 control and 5 lines

37**. Fielder** 10. BC1 – X161-3

**12. BC1 – X187-1** 13. BC1 – X187-2 **14. BC1 - X187-3** 

18**. BC1 – X224-1** 19. BC1 – X224.2

25. BC2- X113-1 26. BC2 - X113-3

	Design #		<b>F1b</b>	الم تعط	510	PC1	PC2	
	Design #	ALL SELFS (S-#)	FIN	/bria	FIC	BCI	BCZ	
	1	BC1-X12-1			Fielder			
	2	BC1-X12-3					-	
	3	BC1-X87-1	Kronos					
	4	BC1-X173-1						
	5	BC1-X27-1						
	6	BC1-X147-1						
	7	BC1-X75-1						
	8	BC1-X88-1						
	9	BC1-X122-1						
	10	BC1-X161-3		MDR031				
	11	BC1-X186-2						
	12	BC1-X187-1						
	13	BC1-X187-2				Paragon	Paragon	
	14	BC1-X187-3						
	15	BC1-X74-1			Paragon			
	16	BC1-X115-1						
	17	BC1-X189-2						
	18	BC1-X224-1	Hoh501					
	19	BC1-X224-2						
	20	R2#2-7		MDR049				
	21	R2#13-1-1		MDB308				
	22	R2#14-1-1		IVIDR308				
	23	R2#16-7						
	24	BC2-X65-1						
	25	BC2-X113-1						
	26	BC2-X113-3		MDR031				
	27	BC2-X150-1						
,	28	BC2-X150-2						
	29	BC2-X150-3						
								4

## What's next ?

Ear morphology
Yellow Rust assessments on flag leaf and 3 top leaves
Aphid infestations ? Specifically R2#2-7 vs MDR049
Flag leaf size
Leaf senescence
Total number of flowering tillers / plant
Plant heights
Grain harvest
Grain shape

Prepare the seed for the 3<sup>rd</sup> wheat – take-all trial to be drilled Oct 2020

### 7<sup>th</sup> July 2020 – Field trial 40 days old



### **Questions for the Breeders**

- What is the consensus for introgressed plant labelling ? To help the dissemination of seeds
- When to start the SSD ?

- Which plants / lines to nominate for DFW Breeders Observation Panel ?



### Many thanks to ....



#### Glasshouse staff – Jill Maple, Fiona Gilzean & Tom Yaxley - Phase 1 lockdown

Field staff - Chris Mackay, Ben Flannery - Phase 1 & 2 lockdown

**Statistician** – Suzanne Clark - Phase 1 lockdown

Gail Canning – location of seed stocks in the seed store and advice on GS scoring - Phase 1 & 2 lockdown



### **Resilience to foliar pathogens**

Kim Hammond-Kosack Vanessa McMillan Gail Canning Deborah Wright



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WGIN MM 8<sup>th</sup> July 2020





- To explore whether *mlo* mediated resistance to powdery mildew is also effective against other fungal pathogens
- To explore the genetic basis of Yellow Rust resistance in two Watkins lines





#### **Powdery Mildew**



bioscience for the future

#### TILLING wheat for mlo mediated mildew resistance



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

Johanna Acevedo-Garcla<sup>1</sup>, David Spencer<sup>1</sup>, Hannah Thieron<sup>1</sup>, Anja Reinstädler<sup>1</sup>, Kim Hammond-Kosack<sup>2</sup>, Andrew L. Phillips<sup>2</sup> and Ralph Panstruga<sup>1</sup>\*





Advancement of Plant Innovation

2019/2020 mlo wheat trial – update on progress

Finally drilled in late March 2020 - 72 plots – identical design to 2018/19 trial

**Established very well** 

Main disease Yellow Rust - scored 3 times on Flag leaf and top 3 leaves

Data gone to statistics dept for analysis



#### Double *ml*o line 4-1-2 was again the most susceptible line in 2020 trial

Trial 1 -2019

19/R/WW/1925: Average combined Yellow Rust score for leaf 3 and leaf 4



### Artificial inoculations with Fusarium done on 15<sup>th</sup> June 2020 (1 week out of Phase 1 lockdown)

**Fusarium inoculations – 15th and 19th June** 



Image DJI\_0174

16<sup>th</sup> June 2020 , Andrew Riche

Scored 20 ears per plot at 21 days - 6<sup>th</sup> and 10<sup>th</sup> July



#### Trial 2 -2019

**19RWW1926:** Fusarium graminearum infection



★ 🛛 = double



- To explore whether *mlo* mediated resistance to powdery mildew is also effective against other fungal pathogens
- To explore the genetic basis of Yellow Rust resistance in two Watkins lines W 733 – dominant resistance W786 – recessive resistance
   High disease in 2020 trial and both Watkins lines still provide high levels of resistance
   Evaluated a larger F<sub>2</sub> population and more F<sub>3</sub> lines





### Many thanks to



Wheat *mlo* project and Watkins YR project Gail Canning Deborah Wright Jessica Hammond (Plant Pathology Apprentice)

Martin Urban - Fusarium inoculations 2019 - Tania Chancellor (2<sup>nd</sup> year PhD student) 2019 - Vanessa McMillan

#### Statistics Rodger White, Stephen Powers and Suzanne Clark

#### **RRes Farm and glasshouse staff**



Wheat Genetic Improvement Network



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# Aphids and BYDV

# Resistance Screening and Diversity Trial Update

Lawrence Bramham 8<sup>th</sup> July 2020







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# Aphid/BYDV Resistance Screening

# Resistance Screening

- Germplasm screened for aphid/BYDV resistance:
  - 20 wheat cultivars from WGIN diversity trial
  - 17 hexaploid lines from the core Watkins landrace collection
  - 33 synthetic wheat lines developed by NIAB EMR
  - 3 additional commercial cultivars (Hereward, Paragon and Solstice), included as control material throughout all testing

# Screening Strategy

 7-day old seedlings (10 per line) challenged with 5 x *Rhopalosiphum padi* reared on BYDV-PAV infected wheat



# BYDV Assay

- Mid/high-throughput assay developed at Rothamsted (Martin Williamson)
  - designed for use with aphids
  - working for <0.5 cm<sup>2</sup> foliar

samples

- `TaqMan'/qRT-PCR capable of identifying both the presence and strain of BYDV
- All  $1^{st}$  leaf samples processed
- 2<sup>nd</sup> leaf samples underway



# BYDV Assay Results

## 20 wheat cultivars from WGIN diversity trial (2019):



### • 17 Watkins lines:

Watkins core	Number of <i>R. padi</i> /BYDV-			Number of unchallenged		
collection	challenged samples			control samples		
designation	R	Ι	S	R	Ι	S
WATKINS_0007	10					1
WATKINS_0032	7			2		
WATKINS_0034	8			3		
WATKINS_0042	9			2		
WATKINS_0044	5	1		3		
WATKINS_0045	9			2		1
WATKINS_0079	9			3		
WATKINS_0103	7			2		
WATKINS_0110	8			2		1
WATKINS_0126	6			2		
WATKINS_0127	5			2		
WATKINS_0139	7			3		
WATKINS_0141	6			2		
WATKINS_0145	7			3		
WATKINS_0160	8			2	1	
WATKINS_0468	7	1		2	1	
WATKINS_0729	8			3		

- R = Resistant
- I = Intermediate
- S = Susceptible

- No BYDV detected in 11/17 of 1<sup>st</sup> leaf samples
- BYDV detected within unchallenged control material, suggesting insecticide treatment was <100% effective</li>

• 33 synthetic NIAB EMR lines (table 1 of 2):

- R = Resistant
- I = Intermediate
- S = Susceptible

 8/33 lines with no BYDV detected in 1<sup>st</sup> leaf samples

Synthetic	Number of <i>R. padi</i> /BYDV-			Number of unchallenged			
hexaploid wheat	challenge	ed sample:	S	control s	samples		
designation	R	Ι	S	R	Ι	S	
NIAB_SHW_012	9	1		3			
NIAB_SHW_018	3	1	1	3			
NIAB_SHW_027	6	1		2			•
NIAB_SHW_028	7	2	1	1			
NIAB_SHW_029	7			1			
NIAB_SHW_030	10			3			
NIAB_SHW_031	5	1		3			
NIAB_SHW_035	7			1			
NIAB_SHW_036	1			1			
NIAB_SHW_042	8		1	2			
NIAB_SHW_051	6	1		2			
NIAB_SHW_054	7			3			
NIAB_SHW_071	5	1	1	1			
NIAB_SHW_072	8			3			
NIAB_SHW_073	6	3		3			
NIAB_SHW_076	7	1	1	1			
NIAB_SHW_077	6		3	3			

• 33 synthetic NIAB EMR lines (table 2 of 2):

- R = Resistant
- I = Intermediate
- S = Susceptible

- 8/33 lines with no BYDV detected in 1<sup>st</sup> leaf samples
- BYDV detected within some unchallenged control material

Synthetic	Number of <i>R. padi</i> /BYDV-			Num	Number of unchallenged		
hexaploid wheat	challenged samples			contr	ontrol samples		
designation	R	Ι	S	R	Ι	S	
NIAB_SHW_080	7			2		1	
NIAB_SHW_082	8		1	3			
NIAB_SHW_083	7	1		3			
NIAB_SHW_084	5			1		1	
NIAB_SHW_085	5	1		1	1		
NIAB_SHW_086	4	1		3			
NIAB_SHW_087	6			2	1		
NIAB_SHW_090	11	1		2	1		
NIAB_SHW_091	8		2	3			
NIAB_SHW_093	9			3			
NIAB_SHW_094	7		2	3			
NIAB_SHW_095	7	1	1	3			
NIAB_SHW_096	8	1		1			
NIAB_SHW_099	7		3	3			
NIAB_SHW_137	4		1	2			
NIAB_SHW_138	9			3			

### • 3 commercial control cultivars:



- R = Resistant
- I = Intermediate
- S = Susceptible

 Consistent indication that cv. Solstice exhibits some level of BYDV and/or aphid resistance

- 2<sup>nd</sup> leaf samples of the three control cultivars processed since ease in lockdown restrictions
- Quality control failures due to passive fluorescence reference dye, so data not yet robust
- No indication of BYDV within cv. Solstice 2<sup>nd</sup> leaf samples (TBC)



# Diversity Field Trial Aphid Monitoring

- 10 tillers per plot assessed (excl. insecticide-treated plots, total = 180)
- Aphid species (*R. padi*, *S. avenae* and *M. dirhodum*) and their morphs recorded



Wingless 'apterae'

3m

9m

# **Overall Aphid Presence**

- Peak in aphid presence around 28<sup>th</sup> May
- correlates with weather and RRes suction trap data for most aphid species
- Indication of aphid migration to field trial at this time, followed by curtailing reproduction of wingless apterae


# Aphid Presence by Cultivar

- Wingless apterae presenting no clear tendency for survival/higher reproduction on individual cultivars
  - data influenced by tillers with high number of aphids
- dynamic biological system, exploring data but no clear correlations to GS, N or cultivar



# Aphid Presence by Cultivar

- Wingless apterae presenting no clear tendency for survival/higher reproduction on individual cultivars
  - data influenced by tillers wit high number of aphid
- dynamic biological system, exploring data but no correlations to GS, N cultivar
- Variation in preference of winged alates to cultivars

clear



# Winged Alates by Cultivar



- Correlations identified previously:
  - higher N  $\sim$  fewer aphids
  - higher yellow rust ~ fewer aphids

50

 Low yellow rust incidence during aphid peak

40

Winged alate count on 28/05/20

# Geographical Distribution

 Uneven distribution of winged alates across entire field trial, but tendency towards discrete plots during migration

G = Guard plots NA = Unassessed plots

(treated with insecticides)



# Summary

 Preliminary BYDV testing has identified lines with potential sources of resistance

– to be validated in future with 2<sup>nd</sup> leaf samples

 Notable aphid movement captured in WGIN diversity field trial with indication of what may be BYDV vectors' preference towards/against certain cultivars

data to be fully explored, potential for future semiochemical research

## WAK bioinformatics update

### Kostya Kanyuka





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### Wall-associated receptor like kinases (WAKs)

#### Cloned WAK genes implicated in pathogen defence

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)
Stb6	wheat	R gene (Zymoseptoria tritici)
Snn1	wheat	S gene (Parastagonospora nodorum)
Sbs1&2	barley	S gene (Bipolaris sorokiniana)
Rlm9	canola	R gene (Leptosphaeria maculans)

DAMP receptor recognising products of plant cell-wall pectin degradation (WAK1)

broad-spectrum, but partial, quantitative resistance (Htn1, qHSR1, RFO1)

> qualitative pathogen resistance in a gene-for-gene manner (Stb6, Xa4, RIm9)

sensitivity to the necrotrophic effector or the pathogen (Snn1, Sbs1&2)





## Wall-associated receptor like kinases (WAKs)

### Structure of a typical WAK protein



#### WAKs characterised in plants

- Arabidopsis (23)
- Rice (75)
- Poplar (122)

vs

#### WAKs in wheat cv. Chinese Spring

- 406 RLK and 2 RLP genes
- 193 pseudogenes (112 RLKs, and 81 RLPs)
- Total 601 sequences among which 518 are RLKs

### **Expansion of WAK gene family in wheat**



## **Clusterisation and subtelomeric location of WAKs**





Features shared with NLRs

## **Clusterisation and subtelomeric location of NLRs**





Steuernagel et al. 2018 bioRxiv

## Are WAKs important players is Septoria resistance?

Gene/QTL	Chromosome	Size of the interval (bp)	Number of WAKs	Candidate WAKs
Stb2	1BS	3,753,304	0	
Stb3	7AS	36,017,764	1	TaWAK530
Stb4	7DS	166,669,925	14	TaWAK557-TaWAK570
Stb5	7DS	96,468,653	13	TaWAK557-TaWAK569
Stb7	4AL	5,073,142	2	TaWAK269, TaWAK270
Stb9	2BL	113,746,383	13	TaWAK88-TaWAK100
Stb10	1D	193,937,871	3	TaWAK44–TaWAK46
Stb11	1BS	73,135,081	15	TaWAK15-TaWAK30
Stb12	4AL	11,685,167	3	TaWAK268-TaWAK270
Stb13	7BL	177,021,373	10	TaWAK547-TaWAK556
Stb14	3BS	2,288,244	8	TaWAK175-TaWAK182
StbSm3	3AS	3,768,577	0	
Stb17	5AL	72,092,188	5	TaWAK293-TaWAK298
StbWW	1BS	73,135,081	15	TaWAK16-TaWAK30
TmStb1	7AS	116,002,803	13	TaWAK518-TaWAK530
QStb.lsa_fb-1A	1A	53,176,139	0	
2DS	2DS	47,410,006	5	TaWAK104-TaWAK108
QStb.lsa_tb-4A	4AL	1,947,740	0	
QStb.lsa_tb-4B	4B	344,665,898	1	TaWAK272
QStb.psr-7D-1	7DS	5,106,019	10	TaWAK559-TaWAK568
7DS	7DS	138,115,519	6	TaWAK569-TaWAK574
7DL	7DL	45,720,815	2	TaWAK576, TaWAK577
QStb.lsa_fb-7D	7DL	3,966,596	1	TaWAK582
QStb.ipk-7D1	7D	440,031,428	6	TaWAK570-TaWAK575
Stb18	6DS	4,765,359	3	TaWAK490-TaWAK492
Stb19	1DS	5,779,654	8	TaWAK35-TaWAK42



## Exome/ promotorome capture of wheat WAKs

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

Captured and sequenced DNA

Mapped captured DNA to the wheat Chinese Spring genome

Allelic variation analysis is in progress



**Chinese Spring** 

- ✓ all exons
- ✓ putative promoters (2-kb sequences)



## WAKs capture analysis

- Baited and sequenced 601 WAK genes (2,638 bait mappings) for 96 accessions. Read data high quality with good coverage: <u>approximately 300X</u> on average for the bated genes
- Stringently mapped sequencing reads end-to-end using using Bowtie2 & filtered (Q30)\* to allow mapping of each read only to a single best possible location in the genome
- Good gene recall for Chinese Spring reference (<10 genes absent) although some regions not uniquely mappable with 151 base reads (e.g. conserved kinase)
- Annotated sequence variants called at 22,853 loci (FreeBayes; DP>=10, variant Q >=30)
- Produced a database of gene models & IGV snapshot sets for visualising and exploration of this large genomic dataset
- Clear patterns of gene conservation and haplotype variation across accessions
- Distance between Chinese Spring reference & bait varieties is an obstacle to definitive absence calls in divergent accessions (other [annotated] references pending)

\* These, together with the CS WAK genes annotations are available for sharing with breeders and academics. The data could be visualised using IGV and used for example for development of new SNP-based markers (e.g. KASP markers).



### Some WAKs are very conserved



#### Single SNP in promoter and no SNPs in exons



## Some WAKs are very conserved

#### TaWAK573 & TaWAK574

	+ FE					1,633 bp					
	ABrosc.	203,940,000 hp	203,844,090 bp	200 340,000 tip	203 348,903 kp	1	283,847,080 tip	283,348,089 tap	203,348,909 bp	200,800,800 Bp	
AlGenes		K K K K K K K TreesCS7D020	10 4 4 4 4 4 1 241700.1				* * * * *	* * * * *	TravesCS7D020241100.1		
WAK_Exons		TaWAKS73_7D	TaWAK573_7D				TaWAK574_7D	-	TaMAK574_70	TaWAK574_7D	2
WAK_Promotors				TaWA	k573_7D_promoter	)					
Mappability151	a cont							11 A 12 A 14			
Bulgaria_8m Coverage											
Sullivan_HH3bam Coverage	p-34										
Chinese_Spm Coverage	p-aq										
Chevignonm Coverage	p-3q										
Penomen_HHm Coverage	p-34										-
Filon_HH3Vm Coverage	p-34										
Gotik_HH3Vm Coverage	p-30										
Grapeli_HHm Coverage	p-24										
Rockefellerbam Coverage	p-aq										
Watkins_42m Coverage	p-sq										
Watkins_61m Coverage	p-34										
Avalon_HH3m Coverage	p-34										
Bobwhite_Hm Coverage	p-34										
Cordiale_Hm Coverage	p-34										
KWS_Barrelm Coverage	p-34										
Riband_HH3m Coverage	P-34										



IGV

snapshot

No SNPs at all

## Some WAKs show high level of sequence variation



A total of 30 SNPs in this gene Is this the same gene or a new gene?

ROTHAMSTED RESEARCH

## Some WAKs show presence/absence variation



Gene is absent in 8/19 wheat varieties



Is this real or do we have some mapping issues?

## Snn1 also shows PAV in the investigated wheats



Snn1 confers susceptibility to
strains of the fungus
Parastagonospora nodorum
that produce effector SnTox1



Snn1 appears to be absent in 48/94 analysed bread wheat

## Validation of PAV for Snn1 in wheat

Snn1 confers sensitivity to the cognate P. nodorum "toxic" effector protein SnTox1

SnTox1 sensitivity data for the 48 varieties potentially missing Snn1:

Varieties No.	Tox1 sensitivity score				
38	0				
3	0.125				
7	0.250				



Conclusion: all wheats identified as missing Snn1 through WAKs capture are insensitive to SnTox1, as expected



## Snn1 may also show copy number variation (CNV)



Some wheats appear to carry more than one <u>non-identical</u> *Snn1* copy

RESEARCH

## **Snn1** shows PAV and CNV in wheat germplasm



Chromosome level NRGene RefSeq genome assemblies for 9 bread wheats and 1 spelt wheat Chromosome level W2RAP (EI) genome assemblies for 4 bread wheats and 1 durum wheat

Arina	absent
Claire	absent
Landmark	absent
Lancer	present
Kronos	present
Julius	absent
Cadenza	present
Jagger	present – 2 copies
Mace	absent
Norin 61	present – 2 copies
Paragon	absent
Robigus	absent
Spelt	present*
Stanley	present – 2 copies
SY Mattis	absent

Using our current WAKs capture dataset it may be impossible to resolve sequences of duplicated WAKs



Dan Smith Rob King Mike Hammond-Kosack Kim Hammond-Kosack

#### Wheat Breeders

Ruth Bryant (RAGT) Nick Bird (KWS) Matt Kerton (DSV) Finn Borum (Sejet) Simon Berry (Limagrain)

International Wheat Genome Sequencing Consortium Thank you!

INRA@

Cyrille Saintenac

James Cockram

NIAB

David Lopez

### WGIN Scientific Outreach Jan – July 2020

Seedstor at the GRU – for access to all WGIN seedstocks

Joint GINs website – June 2020

Virtual Cereals ppt – June 2020

Crop Production Magazine – July 2020 WGIN, PCGIN and OREGIN

Open Access Government site – advertising the virtual Stakeholder meeting 11-12<sup>th</sup> Nov 2020

### All WGIN generated seed stocks available from Seedstor at the GRU

### https://www.seedstor.ac.uk/search-panel.php



### https://www.seedstor.ac.uk/search-browsecollections.php



### The new joint GINs website: https://defracropgenetics.org

#### **Tested in May and went live in June 2020**



QR for the site

Scaleable for use on any mobile device in portrait or landscape modes



### News Publications per GIN

Brief project descriptions + dates for stakeholder meetings

### Link-outs to the four individual GIN websites

#### Developer : James Seager (RRes -BCP PHI-base team), software is WordPress

### Virtual Cereals event – 11<sup>th</sup> – 12<sup>th</sup> June 2020







*T. monococcum* as a source of take-all resistance - Video WGIN



11 Jun 2020

#### 8:00 - 13:45

**Plant Breeding Innovations** 

#### JOIN WEBINAR

#### Chaired by Anthony John, Department of International Trade

This session explores the opportunity that plant breeding techniques such as gene editing and sequencing, alongside increasing collaboration between researchers and industry, offer in creating crops and varieties that are much fitter for a future that aims to be low in carbon and pesticide usage.

- Kim Hammond-Kosack, Rothamsted Research reveals a bold new resilient world in which breeding technologies will deliver resistance to aphids, take-all, septoria and slugs in the future
- James Brosnan, chair of the International Barley Hub, which is galvanising the research community and supply chain to address the big issues of resilience and climate change, talks about the innovations coming barley's way
- **Cristobal Uauy** gives an insight into CRISPR gene editing technology and how it can be used to produce new varieties with benefits to farming and the environment.
- Rob Hiles, Syngenta looks at the potential benefits of hybrid wheats, which are getting closer to becoming a commercial reality.

#### BASIS POINT AVAILABLE

https://www.cerealsevent.co.uk/cereals-2020-live-webinar-timetable

New germplasm, new tools, new traits, new genes

WGIN4 Core Project March 2018 – March 2023

www.WGIN.org.uk



333 in the audience on the day100 plus viewings since

### Kim Hammond-Kosack



Department for Environment Food & Rural Affairs





11<sup>th</sup> June 2020

### CPM magazine special issue on Crop Genetics – July 2020



#### **Could the UK's**

"extraordinary" plant-science sector be limbering up for an integral role in the genetics revolution that will deliver the farming of the future to communities the world over? CPM explores the opportunities

By Tom Allen-Stevens

As the Government faced criticism for appearing to slam shut one door on the path to prosperity for UK Agriculture, it may just have opened another.

At Cereals Live last month, farming minister Victoria Prentis explained why Government ruled out measures in the Agriculture Bill to prevent farming standards being undermined through imports (see full report on p34). But she struck a different note on gene-editing, highlighting that Defra doesn't agree with EU laws that treat the new plant-breeding technology (NPBT) as genetic modification.

"We've pushed for many years for the EU to come to the place we're in," she said. "We're committed to taking a more scientific approach and we recognise the potential for

Agriculture Bill, this is definitely an important area for Defra to be engaging with over the course of the next 12 months."

This follows the tabling of an amendment to the Agriculture Bill in the House of Lords by former CLA president and Countryside Agency chair Lord Cameron. The amendment on agricultural research would allow the Defra secretary of state to change the way the Environmental Protection Act treats breeding techniques "where nucleic acid changes could have occurred naturally or through traditional breeding methods." If the amendment's carried, any changes would only be made following public

#### **Reasearch programmes**

to England.

consultation and would be restricted

Lord Cameron said he wanted the Bill to put forward a "clear message" that the Government will move forward to allow gene-editing in research programmes. "This is a way of speeding up the natural methods of farm breeding to ensure that we can improve the environmental and nutritional outcomes of feeding our ever-expanding human population, both at home and -more particularly, as far as I am concerned in the developing world," he told the House during the Bill's second reading last month.

So just what is the prospect for UK plant-science research and what role could arable farmore have? Moreover with a

This CPM genetics special explores the prospects for UK-based plant breeding over the next 11 pages. Since 2003, four Genetic Improvement Networks (GINs) have formed the focus of Defra's activity in this area, for

wheat, oilseeds, pulses and vegetables, The department's awarded them a total of £5.5M for a five-year programme of activity up to 2023.

The wheat network, WGIN, for example, has £1.7M of this, with its main aim being to improve the resilience of the UK wheat crop through genetics. About 70% of its effort goes towards trait discovery while the rest is spent on developing genetic and genomic resources, available licence-free to commercial breeders to introduce into their elite lines.

One new aspect of the 17-year-old

programme is that there's now an alliance

with AHDB to strengthen direct links with

farmers. Traits being explored include

nitrogen use efficiency, resistance to

lodging, slugs and BYDV, as well as

resistance to septoria and yellow rust.

Centre, for example, with novel lines

showing up to 20% improvement.

Work on quality traits includes research on

specific weight undertaken at John Innes

Drones with high-periormance cameras

are being developed for faster phenotyping.

while one of the jewels in WGIN's crown is

the Watkins collection - a unique set

of 1200 lines from 31 countries never

proviously used in modern breeding

WGIN



agement, with emphasis on those that

nd market pressures that are commonly long been the backbone of cited as putting increasing strain on the offsoed sector, and these factors haven a Defra-funded breedin cone unnoticed. "Everyone is acutoly aware rogramme that has of how much of a priority cabhana stor fea beelle is," says AHDB research and responded to the challenge knowledge exchange manager Dhan facing oilseed rape growers. CPM explores the AHDB doesn't contribute to funding, he explains, but does got involved in the many programme's current focus spin off research projects that have com about through OREGIN, and sits on the By Tom Allen-Steve stakeholder group. "We're picased to hav onnortunity to influence the programm

although the targets OREGIN has identify Fow crops will take you on the emotional rollercoaster in quile the same way as obseed rape. There's the dazzling display of a crop in full flower, followed by the and the direction taken we fully endorse. I icmonstrates good indi. notes Dhan thick mat of pods with its promise of Alternative approaches harvest booty. And there's the strugglin Broadly, the direction taken is to seek seedlings mercilessly savaged by cabl stem flea boetle as they sit in submiss out atternative approaches to crop surrender in a dry September soil. Could something be done with the genetics to smoothen out the ride? integrate genetics with seed and agronomi chinologies and methods to milligate blob This is one of the key aims of the Oilseed and abidic stresses. 'Previous research had implicated specific genes as being (OREGIN). Set up in 2003 as one of four doltimental to important characteristics o Detra-funded genetic networks, if bring aposeed, so the current phase of OREG logether researchers and breeders in a cludes pro-brooding activities to last collaborative approach to pre-breeding wor prodicted impacts of the loss of those and susception. Priority plant characteristics iones," he explains. This involves the development of new eing investigated include tolerance h insects, viruses and diseases, including mborna storn canker, light leaf spot and clubroot, and improved lerilliser u efficiency

(a) crop production magazine july 202

A characteristic of all GINs, and a unique leature of the wider programme, is that information has always been made publicit mailable licence-free, to guide breeder owards estired trait

"They're not starting from scratch threaders have been incorporating these mprovements since the programme began What's different now are the advances in breeding lochnology. Receders brack genes of interest or areas of the genome as with a trait. Markers help them quickly identify if this gone or set of gones has come through a new cross, and this can reduce the tim it takes to bring a new variety to market CREGIN gives the broaders not only the permokesm that has the interesting trait, bu he markers to help identify it." says Dhan





#### PCGIN



lamental problem

med at putting varie Jack would like to see "aglie alliances" between arable and liveslock producers on farm that will m underpinned by a "professionalisation" of farm-to-farm trading. But he recognises

By Tom Allen-Stever

ropresent a whole tamily of this wonder

produce, but even the most widely grown

peas and beans - are minor crops in the

Jack Walls believes it's a missed opportun

"Putros come with built-in public good.

The EU currently imports around 90% of

so present a dual purpose for society and

its protein requirement, with scybean and

between 2.5-3M lonnes/yr. "EU policy to

soya meal imports into the UK alone

the past 40 years has driven quite a

significant oversupply of energy-dense

grains and that's created a massive deficit

"Sovmeal is very competitively priced and

termors alko," he notes.

UK. NEU agrifood policy dolivery manage

A crop that fixes its own nitrogen from they struggle to compete on the marke the air, is nutrient-rich, with properties with sova and in the rotation with wheat that offer a wide range of estat The challenge for the crop is consistency i health benefits and one that can hav vields and it's clear UK pulse-breeding and a carbon-positive impact in many of its agronomy need fast tracking to offset applications: such a crop should surely b docades of neglect." beraided and championed? Pulse crops are widely considered to

POPO's Slove Policher across that year-on-year variation in yields is a problem opping pulse crops out of the rotation for many. "Particularly in spring-sown crope there's a perception works are variable and inreilable, although there's also evid this is no more the case in pulses then for that's come about through decades of policy other spring-sown crops," he notes

The opportunity for protein substitution is to some adent limited by availability Slove points out - pulses will at best only ever be included one wear in five in the standard UK arabio rotation. "This is an area that gots a lot of rhelpric, but not a lot of action

Detra's response is to fund the Pulse Crop Ganatic Improvement Network (PCGIN). One of four networks, it's a platform that sets out In some the process of crep improvement to legumes in the UK. PCGIN is currently

JUST WHEEL running on a five-year programme, with

of home-grown protein." Jack maintains. (2) crop production magazine july 2020-





Genetics special – The global reach of plant science

Search our ar

### 1) 'Genetics special – The global reach of plant science' http://www.cpm-magazine.co.uk/2020/07/05/genetics-specialthe-global-reach-of-plant-science/

So just what is the prospect for UK plant-science research and what role could arable farmers have? Moreover, with a US/UK trade agreement being discussed, how will UK plant science fit into the new world order, and what support can we expect from government?

This *CPM* genetics special explores the prospects for UK-based plant breeding. Since 2003, four Genetic Improvement Networks (GINs) have formed the focus of Defra's activity in this area, for wheat, oilseeds, pulses and vegetables. The department's awarded them a total of £5.5M for a five-year programme of activity up to 2023.



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WGIN aims to improve the resilience of the UK wheat crop through genetics. For full details see GINs provide shot in the arm

Drones with high-performance cameras are being developed for faster phenotyping, while one of the jewels in WGIN's crown is the Watkins collection – a unique set of 1200 lines from 31 countries never previously used in modern breeding. A range of novel traits is also available through 323 lines of *Triticum monococcum*, a diploid

wheat rarely used in modern breeding – resistance to take all, septoria, yellow and brown rust, mildew and aphids have all been identified.

Similar achievements have been made with OREGIN and PCGIN. But is it enough? "We see an opportunity for a step change in the way we do plant science," says Prof Dale Sanders, director of the John Innes Centre.

2) 'Theory to Field – The OREGIN of resilience?'

http://www.cpm-magazine.co.uk/2020/07/06/theory-to-fieldthe-oregin-of-resilience/

3) 'Pulse Genetics – Reach for the GIN'

http://www.cpm-magazine.co.uk/2020/07/09/pulse-geneticsreach-for-the-gin/ https://www.openaccessgovernment.org/rothamsted-research-strategic-agricultural-science/74522/

Ongoing since May 2019



**Agricultural Sciences section** 

### The Defra funded UK Wheat Genetic Improvement Network, in its 16th year of funding and still going

strong.

This year's virtual WGIN Stakeholders' Meeting will take place on Wednesday and

#### Thursday November 11<sup>th</sup> and 12<sup>th</sup> 2020.

This event is open for registration from September onwards via Eventbrite by visiting the WGIN website (www.wgin.org.uk/) where a copy of the full programme can also be downloaded. This is an opportunity to 'meet' and discuss current and emerging topics in wheat research, genetic improvement through breeding and the latest production methods with wheat scientists based in academia and industry, commercial plant breeders, crop consultant, farmers and farm managers.



Figure 1 The organisational schematic of WGIN4. All red text highlights new additions compared to WGIN3

+ 500 words of text

Mailshot Reach ~120, 000/annum across UK and Europe

- Governments, EU Leaders
- NGO Leaders
- Heads of Large companies