WGIN has now been extended by DEFRA until 2023 – Welcome to WGIN 4!

Contents:

Defra Wheat Genetic Improvement Network (WGIN): Improving the resilience of UK wheat yield and quality through crop genetics and targeted traits analysis

Section 1 Project Background

The UK government is committed to more sustainable agriculture but this vision is facing an ever expanding range of environmental, energy and climate change challenges. Wheat is grown on a larger area and is more valuable than any other arable crop in the UK. The **Wheat Genetic Improvement Network (WGIN)** started in 2003. The overall aim of WGIN is to generate pre-breeding material carrying novel traits for the UK breeding companies and to deliver accessible technologies, thereby ensuring the means are available to produce new, improved varieties. To achieve WGIN's goals, an integrated scientific 'core' was established which combines underpinning work on molecular markers, genetic and genomic research, together with novel trait identification. The programme is managed by a team including representatives of the key UK research groups and breeders. They ensure the programme and its outputs are communicated to the wider scientific and end user communities, via a web site (www.wgin.org.uk), an annual stakeholders' meeting and peer reviewed publications.

The 3rd phase of WGIN (WGIN3), which has now almost ended, was entitled '**Improving the resilience of UK wheat yield and quality through crop genetics and targeted traits analysis**'. This project consisted of four work packages (WPs) (**Figure 1**). WP1 focussed on further enhancing the networking and communication activities. The three inter-connected research work packages (WP2, WP3 and WP4) focussed on exploring a range of high priority traits for the UK wheat crop and then undertaking detailed genetic and quantitative trait loci (QTL) analyses (WP2 and WP4), maintaining and developing new genetic resources for the UK research community (WP3), and testing new tools based on next generation sequencing technologies for their applicability to wheat research (WP4). **This newsletter is thus the last one to be circulated under the WGIN3 banner**, but importantly and happily for all involved, it is **NOT** the last WGIN newsletter (see below).

The funded partners in WGIN3 were the **John Innes Centre (JIC)**, **Rothamsted Research (RRes)** and two sub-contractors, the **Bristol Genomics Facility**, based within the University of Bristol and the company **MYcroarray** (recently renamed as **Arbor Biosciences**), based in Michigan, USA.

All of these partners will also be part of **the next phase** of WGIN, because **Defra has now funded WGIN for a further five years until 2023!**

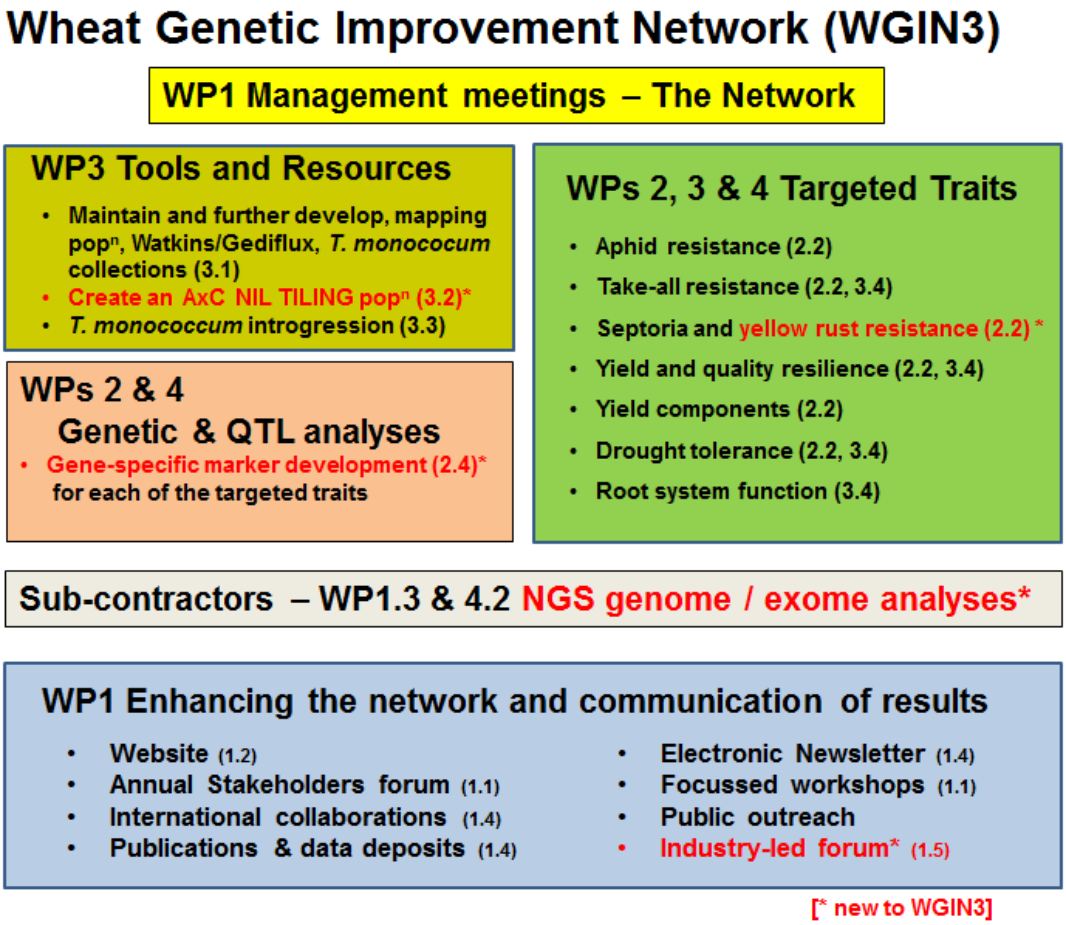

Figure 1: The design of WGIN3 with details of the four work packages (WP)

Section 2 Research Update

Developing New Resources for Wheat Gene Discovery (JIC)

- **A Chromosome Segment Substitution Library (CSSL) for the Avalon x Cadenza (AxC) Population**

WGIN has successfully promoted the AxC doubled haploid population (DH) as the UK reference since 2005 and it is now the most densely mapped wheat population in the world. We having been developing a new unique resource using the AxC DH lines. Details of the concept behind this experiment were presented in the 2017 WGIN newsletter; here we present the latest analysis of the data. We used the Axiom 35K wheat breeders array to screen 94 NILs, from the Cadenza and Avalon recurrent backgrounds. The selected NILs include those with QTL for heading (1B, 1D, 6A, 6B), height (2A, 2D, 3A, 3B, 6A, 6B) and yield (2D, 3B, 5A, 7B, 7D).

	A genome						
	1A	2A	3A	4A	5A	6A	7A
Avalon (44 lines)	10%	20%	95%	80%	90%	95%	70%
Cadenza (50 lines)	95%	95%	95%	75%	95%	95%	85%
	B genome						
	1B	2B	3B	4B	5B	6B	7B
Avalon (44 lines)	95%	20%	95%	90%	95%	95%	60%
Cadenza (50 lines)	90%	90%	95%	95%	95%	80%	75%
	D genome						
	1D	2D	3D	4D	5D	6D	7D
Avalon (44 lines)	30%	20%	80%	?	75%	95%	95%
Cadenza (50 lines)	90%	90%	60%	?	80%	95%	70%

Figure 2: Approximate percentage coverage of substituted segments

Preliminary results of the data were presented in the 2017 WGIN newsletter. When the IWGSC RefSeq v1.0 genomic sequence data becomes officially available, this will allow us to display the data more accurately by showing the position and extent of substituted

chromosomal segments on the physical chromosomes. This data will be displayed on the WGIN website as soon as possible. Analysis of the 35K genotyping data showed that there is very good, though not complete, coverage of substituted Avalon segments in the Cadenza background but that the coverage is not so high in the opposite combination (**Figure 2**).

All the 94 CSSL lines were backcrossed to the recurrent parent and selfed. 57 lines representing the maximum genome tiling path possible (due to coverage and crossing success) have been selected and KASP markers in each substituted segment will be used to screen the BCF_2 to identify lines carrying the desired segment. A lower than expected % of background in some NILs means these lines are already suitable as a resource for donating a single chromosome segment.

Overall these new genetic resources aim to provide every research team in academia or industry working with AxC to focus on their target region using just four precise genotypes, i.e. every combination of Avalon or Cadenza background, with a chromosome substitution from Avalon or Cadenza, subject to the degree of coverage. We believe the availability of these new lines with this high level of genotypic characterisation will open the possibility for completely new experiments and experimental types based on the highly successful AxC DH resource.

For further information on this aspect of the WGIN project contact Clare Lister (clare.lister@jic.ac.uk) or Simon Griffiths at the John Innes Centre (simon.griffiths@jic.ac.uk)

- **Quantifying Agronomic Impact of WGIN Target Genes Using the Paragon NIL Library**

WGIN has been part of an informal consortium developing NILs in the genetic background of the UK spring wheat Paragon. The collection, known as the Paragon Library (PL), was developed at JIC and currently consists of around 350 lines. Please see the attached flyer located at the end of this Newsletter for further information on the PL. We have concentrated on the analysis of a subset of the PL (**Table 1**) and these lines have been included in the PxG drought trials.

The 2017 WGIN newsletter described how this subset was grown beneath the Phenospex laser scanning phenotyping platform in a nitrogen usage trial in 2015-2016 at JIC, in collaboration with Ji Zhou, Earlham Institute (EI), Norwich. The height of the plants was monitored in real time over the growing season. This experiment was repeated in 2016-2017 with a particular focus on the rate of growth of the semi-dwarf lines (RhtB1, RhtD1 and Rht8) compared to Paragon. The results are shown in **Figure 3**.

Table1: Details of the subset of Paragon Library Lines

Description	Source	Comment / Trait
Lr19 Kamb1	Kamb1/Para5-7-7-21-10-2	Alien introgression
Par Mutant 2316b	Par EMS 2316b	Staygreen mutant
Ppd 1x Early	Par (CS 2B) -1- 3a3	Heading
Ppd 2x Early	Par (GS100 2A+CS 2B)-T12 F4- 3b1	Heading
Ppd 3x Early	Par (GS100 2A+CS 2B+Son64 2D)-T10 B10- 3b16	Heading
Ppd KO 3x	Par (Norstar+Gamma 319c) 3c-11	Heading
Rht 8 Mara	H14 Nor N Med Irr-9	Height
Rht B1 Robigus	Rob/Par^{7}144-5-171-2-8 Rob	Height
Rht D1 Alchemy	Alc/Par^{7}139-10-179-1-2 Alc	Height

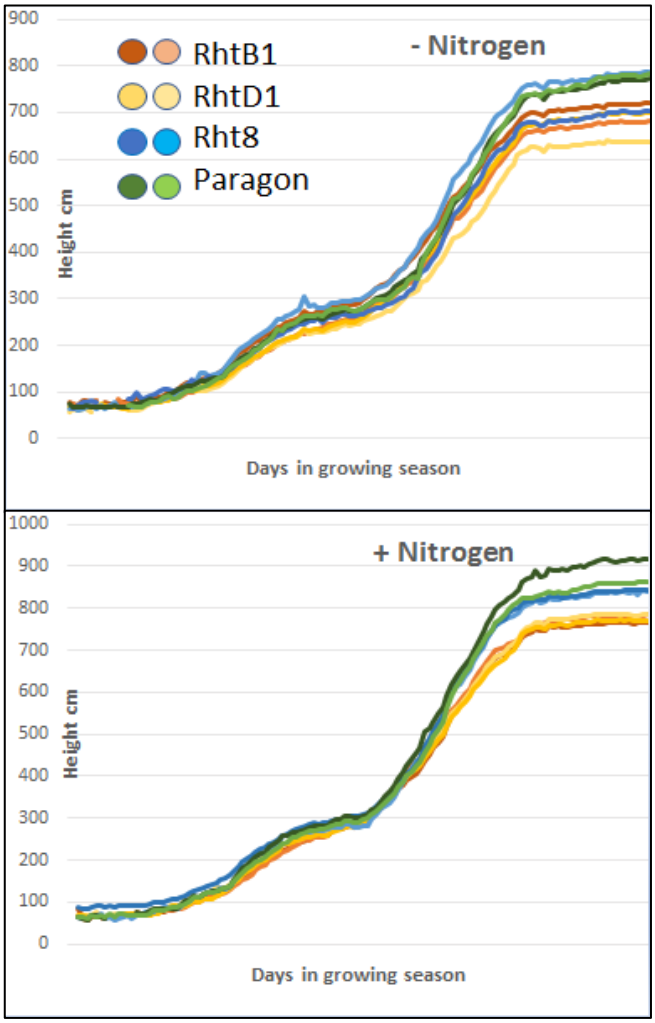

Figure 3: Real-time growth rates of *Rht* lines and Paragon, with and without nitrogen

These data provide new insights into the rates of wheat growth and how this varies in some semi-dwarf mutants. To continue this work, a John Innes Centre Institute Strategy Funding (JIC ISF) has been awarded for RNA sequence analysis and gene network modelling. A large proportion of the current Paragon Library has been genotyped on the Axiom 35K wheat breeders array and the genotyping data will be made available on the WGIN website as soon as possible. Seed will be generated from the genotyped plants and deposited in the Genetic Resources Unit and the JIC and should be available on request in summer 2018.

For further information on this aspect of the WGIN project contact Clare Lister (clare.lister@jic.ac.uk) or Simon Griffiths at the John Innes Centre (simon.griffiths@jic.ac.uk)

- **Foundations for a new generation of segregating populations for studying yield stability in the UK**

Part of WGINs remit is to generate new populations, specifically targeting UK yield stability. To fulfil this objective, Simon Orford, the derived germplasm specialist at the Germplasm Resource Unit in Norwich, made crosses between many Recommended List varieties to generate F_2 seed (**Table 2**). A few selected lines have been taken to the F_4 or F_5 and all this seed is available on request from the Genetic Resources Unit (GRU), JIC. The rationale for the selection of these crosses is NOT to produce new varieties but to highlight genetic variation between RL groups and breeding programmes that has been exploited in recent years to produce the best UK varieties.

Table 2: New populations generated in WGIN to target UK yield stability

Female	Male	R_value Diversity target	R_value Diversity target	RL value Diversity target	Gene-ration
Grafton	Conqueror	lodging	powdery mildew	eyespot	F2
Grafton	Revelation	heading	brown rust	fusarium	F2
Beluga	Cordialle	protein	Hagberg	TGW	F2
Scout	Denman	lodging			F2
Revelation	Gallant	heading	fusarium		F2
KWS Sterling	Alchemy	height			F2
KWS Santiago	Scout	yield	eyespot		F2
KWS Santiago	Solstice	yield			F2
KWS Santiago	Gallant	Hagberg	market share		F5
KWS Kielder	Scout	yield			F2
KWS Kielder	Einstein	yield			F2
KWS Gator	Gallant				F2
KWS Gator	Revelation	*Septoria tritici*			F2
KWS Croft	Scout	lodging			F2
Icon	Skyfall				F2
Horatio	KWS Gator	GS31			F2
Grafton	Solstice	height	eyespot		F5
Gallant	Invicta	heading	market share		F2
Gallant	KWS Kielder	market share			F2
Einstein	KWS Santiago	yield			F2
Cougar	Claire	yield untreated			F2
Cordialle	Revelation	heading	brown rust		F5
Cordialle	Crusoe	GS31	market share		F4
Cordialle	Alchemy	height			F2
Cordialle	Invicta	heading			F2
Conqueror	Scout	lodging	eyespot		F2
Claire	Revelation	yield untreated			F2

At the other extreme, very diverse populations exploring new phenotypes of potential interest have been generated. Examples of this are the 3N alien introgressions (from *Aegilops uniaristata*) into Chinese Spring. These three lines show a prolific root phenotype and aluminium tolerance. Aluminium toxicity primarily affects the division and elongation of the root apex so a robust root system might be expected in plants exhibiting aluminium tolerance. The lines have been crossed into several winter elite lines and one of the 3N introgressions into Cordiale was drilled in 1m^2 plots in autumn 2017 (**Figure 4**). Although aluminium toxicity is not a major problem in UK agriculture, it is hoped that rooting characteristics of these lines might carry benefit for UK breeding. In WGIN 4, the effect of the ±3N alien introgressions on responses to Take-all root infections will be explored in 3rd wheat crop situations.

Figure 4: 3N Introgression from *Aegilops uniaristata* (left) into Chinese Spring and subsequently crossed into Cordiale (right)

For further information on this aspect of the WGIN project contact Clare Lister (clare.lister@jic.ac.uk) or Simon Griffiths at the John Innes Centre (simon.griffiths@jic.ac.uk)

- **Dissecting UK Drought Tolerance in the Paragon x Garcia Population**

Wheat is susceptible to drought at the start of stem extension (stage 31) when grain number is being determined. In the last seven years, drought in East Anglia has occurred five times during April, which coincides with this vulnerable period. We have been looking for drought-tolerant (DT) characteristics in RILs generated from a cross between Paragon (UK spring wheat) and Garcia (bred for drought conditions in S. Europe). The 177 Paragon x Garcia (PxG) RILs identified as Ppd-sensitive, along with nine lines from the Paragon Library (PL), plus Paragon and Garcia controls and Soisson marker line, totalling 200 lines, were selected for the Drought Trials. The trials consisted of two randomised reps each of all lines in Not Irrigated (NI) and Irrigated (IR) 6 m^2 plots. The first PxG Drought Trial (DT) was drilled in autumn 2015 but there was so much rainfall in spring 2016 that the ground was waterlogged for the whole of April and into May! In autumn 2016 we carried out the second drilling of the trial, with our fingers crossed for a drought, but of course you have to be careful what you wish for… As all farmers and plant breeders know to their cost there was a severe drought in April 2017 and therefore very difficult conditions for growing wheat, and other crops. **Figure 5** shows the soil water content at the John Innes Centre's Church Farm at Bawburgh, Norfolk, measured using the ML3 probe from Delta-T (www.delta-t.co.uk/product/ml3).

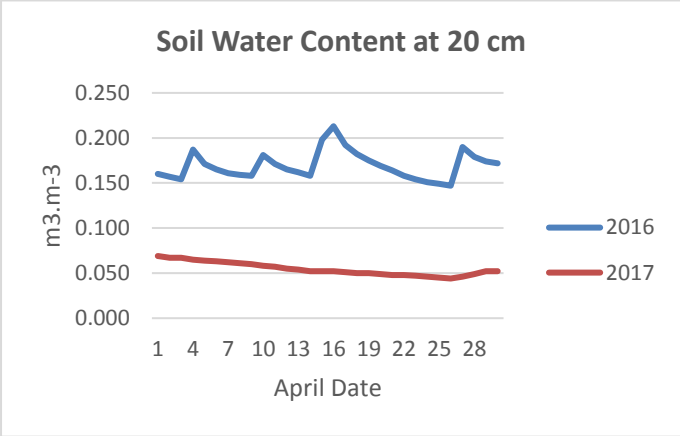

Figure 5: Soil water content of Drought Trial in April 2016 and 2017

The April drought however was exactly the conditions we needed for a successful trial, although the continuing drought did require us to eventually irrigate the NI plots too or we could have lost the trial. Regular photographing of the trial (at 110 m, by Simon Orford) from a DJI Phantom 3 Professional drone very clearly shows the progression of the drought and its effect on the trial (**Figure 6**).

Figure 6: Aerial photographs of the PxG drought trial in 2017, dates shown (NI=non-irrigated, IR=irrigated)

Multiple traits were scored, measured or observations made and much of this phenotype data was put together with genotype data for QTL mapping. **Table 3** shows the QTL's identified in both the 2016 and 2017 trials. While many QTLs are common, including those for known loci for flowering and height, and also coming from Paragon, there are clearly at least two QTLs coming from Garcia which increase yield (on 2B and 1A), with the QTL on 1A being specific to the NI plots in 2017.

Table 3: Summary of the QTLs identified in the PxG drought trials of 2016 and 2017

	2016			2017		
	Linkage group	%Expl. Var	High value allele	Linkage group	%Expl. Var	High value allele
Booting NI	2B	10.0	Gar	2B	9.3	Gar
	2D	21.5	Par	2D	21.2	Par
	4D	6.4	Gar			
				5B	11.0	Par
	7A	17.0	Par	7A	10.8	Par
Booting IR	2B	9.5	Gar	2B	6.2	Gar
	2D	14.9	Par	2D	53.9	Par
	7A	19.7	Par	7A	14.9	Par
Heading NI	2B	10.3	Gar	2B	6.6	Gar
	2D	22.3	Par	2D	21.3	Par
				5B	12.0	Par
	7A	17.1	Par			
Heading IR	2B	9.8	Gar	2B	4.7	Gar
				2D	38.4	Par
	7A	15.3	Par	7A	21.1	Par
Height NI	1A	4.4	Gar	1A	4.8	Gar
	3B	3.7	Gar			
	4D	62.3	Par	4D	62.3	Par
Height IR	1A	6.4	Gar	1A	5.1	Gar
	3B	4.0	Gar	3B	3.9	Gar
	4D	59.6	Par	4D	59.3	Par
				7A	5.4	Par

2B and 1A candidates for increased yield in IR and NI
In **2017** 1A for TGWT is specific to the NI plots

	2016			2017		
	Linkage group	%Expl. Var	High value allele	Linkage group	%Expl. Var	High value allele
Yield NI				2B	17.7	Gar
	7B	16.8	Gar			
Yield IR	NO QTLs DETECTED			1A	11.4	Gar
				2B	17.0	Gar
Specific Weight NI	2B	9.0	Gar	2B	11.3	Gar
	3A	6.1	Par			
	4D	29.2	Par	4D	33.7	Par
	5A	2.5	Gar			
				7D	5.2	Gar
Specific Weight IR				1A	5.0	Gar
				2B	9.4	Gar
	4D	38.1	Par	4D	33.0	Par
	5A	6.8	Par			
	5A	7.0	Gar			
TGWT NI	1A	18.2	Gar	1A	14.6	Gar
	3B	4.5	Gar			
				4D	16.6	Par
	5A	6.3	Gar	5A	5.0	Gar
				5B	8.4	Gar
				7A	12.3	Gar
TGW IR	1A	15.0	Gar			
	4D	5.2	Par	4D	18.7	Par
	5A	6.5	Gar	5A	7.1	Gar
	5B	14.2	Gar	5B	9.2	Gar
				7A	8.2	Gar

Analysis of the yield data shows that lines carrying these loci performed better than Garcia under drought conditions and had a higher yield in NI than IR (**Figure 7**).

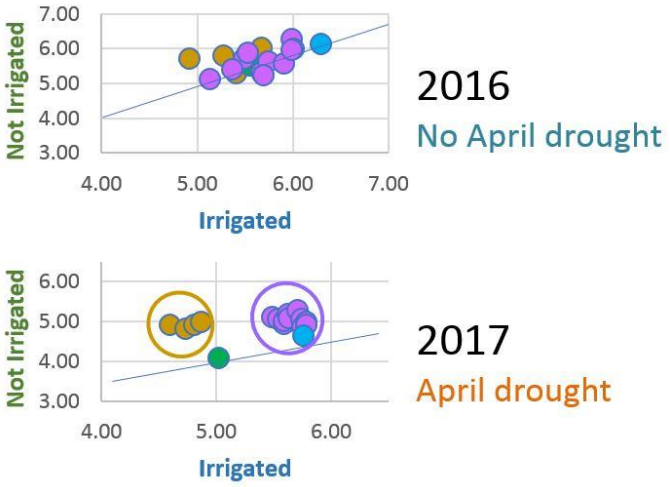

Figure 7: Yields from the IR plots plotted against the yields from the NI plots in 2016 and 2017. The mustard coloured lines carry the 1A QTL and have the highest yield in NI compared to IR. The purple lines carry the 2B QTL and have a higher yield than Garcia in NI. Paragon=green and Garcia=blue.

For further information on this aspect of the WGIN project contact Clare Lister (clare.lister@jic.ac.uk) or Simon Griffiths at the John Innes Centre (simon.griffiths@jic.ac.uk)

- **Nomination of WGIN drought QTL for the Designing Future Wheat Breeder Toolkit**

Designing Future Wheat (DFW) is a cross Institute Strategic Programme funded by the Biotechnology and Biological Sciences Research Council (BBSRC). When new and potentially useful genetic variation is identified, DFW uses a mechanism called the 'Breeders Toolkit' to transfer the genes/alleles of interest to UK varieties. More information on this process is available at http://wisplandracepillar.jic.ac.uk/toolkit.htm.

The composition of the Breeders Toolkit Committee is shown below in **Figure 8**.

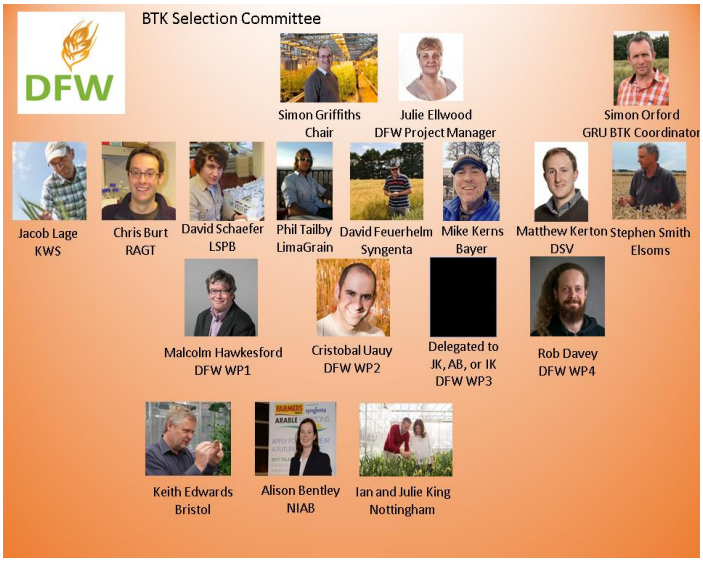

Figure 8: The Breeders' Tool Kit Selection Committee

The Paragon x Garcia yield QTL described above were successfully nominated for inclusion in the Toolkit in February 2018. This means that new lines carrying these Garcia alleles will be produced by DFW in collaboration with the Germplasm Resource Unit (GRU) based at the John Innes Centre in Norwich. If the lines produced continue to display improved performance under drought conditions they will become Breeders Toolkit lines and multiplied for assessment at multiple commercial and academic sites across the UK.

Nitrogen Use Efficiency (NUE) and Quality QTLs linked to NUE (RRes)

- **WGIN Diversity Experiment – investigating key components of yield**

The WGIN Diversity trial has been grown at Rothamsted each year since 2004. The field experiment currently has 30 varieties of wheat grown at four levels of applied nitrogen fertilisation (0, 100, 200 and 350 kg N/ha). A core set of varieties, going back to the 1960s, is grown each year; then, from a set of additional varieties, one or two current varieties are added most years, and one or two outclassed varieties dropped. The experiment is used to investigate variety x N interactions, nitrogen use efficiency and yield stability as well as providing a useful resource, including plant and soil samples, for other research projects and student projects.

For the past four years, the plots have been sampled at anthesis, and as part of this activity fertile ears have been quantified along with thousand grain weight at harvest. This has provided new data on yield components, and how these are affected by nitrogen availability.

One way of considering wheat yield is as follows:

Yield (t/ha) = **Thousand Grain Weight** (TGW) (g) x **grains/ear** x **ears**/m^2/100

We have measured yield, TGW and ears/m^2, and can therefore estimate grains/ear. **Figure 9** shows the ear population plotted against grain yield. The data are the means of three years (2014, 2015 and 2016). The plots are sown at 350 seeds/m^2, and therefore it appears that on average each plant has 1 – 2 ears at harvest. This number is affected by nitrogen with far fewer ears at zero N, through to just a small increase between 200 and 350 kg N. Yield increases with ear populations up to around 500 ears/m2, after which yield levels off. However, within the Rothamsted trials, few varieties achieve an average higher than 550 ears/m^2.

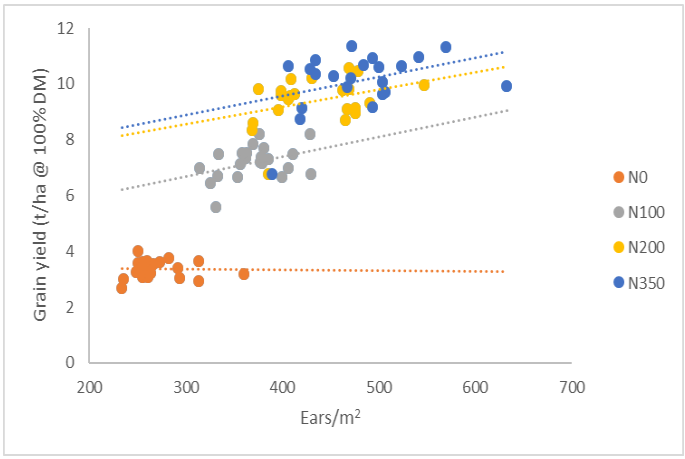

Figure 9: The effect of ear population on grain yield, shown is mean data across the three years 2014-2016. Four levels of nitrogen fertilizer use, i.e. 0, 100, 200 and 350 kg N/ha.

Nitrogen also affected the number of grains per spike (**Figure 10**), with far fewer grains at zero N and little difference between N200 and N350. Yield was also related to grain number. For example, at the N200 level, the variation in yield by a factor of 1.57 (6.76 – 10.58 t/ha) was similar to the variation in grain numbers, which varied by a factor of 1.39 (40.71 – 56.79 grains/ear). However, there

was quite a lot of variation, with some varieties having relatively high grain numbers but low yield: Avalon, a popular variety in the 1980s, had 52 grains/ear but only yielded 8.60 t/ha. This may be because older varieties lack the yield potential of modern varieties, and even though they can set high grain numbers these varieties do not achieve the highest yields.

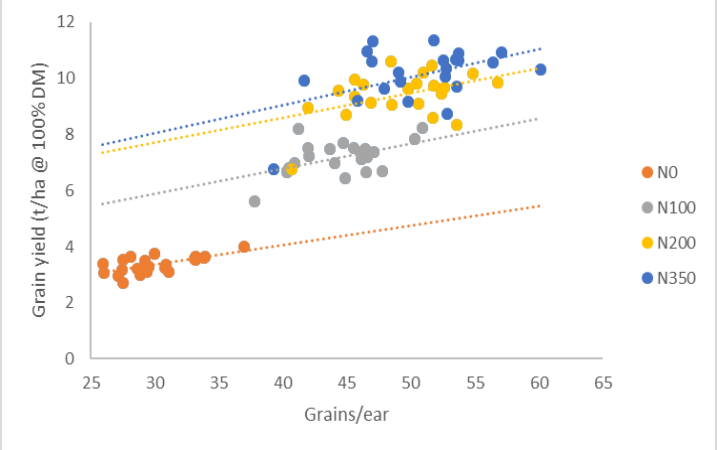

Figure 10: The effect of grains per ear on grain yield in the WGIN Diversity field experiments (2014-2016).

Nitrogen had no effect on TGW: the mean TGW at N0 was 43.7g, N100 44.5g, N200 44.7g and N350 44.1g; TGW also had very little effect on yield.

If the data on grains/ear and ears/m^2 are combined, producing grains/m^2 (**Figure 11**), then a very clear effect of grain number on yield can be seen. Grain yield increases linearly up to 25 000 grains/m^2.

There is some evidence from the data that wheat breeding has enabled varieties to maintain very slightly higher ear numbers and grains per ear and this is part of the reason for the increase in yield seen over the period covered by the varieties grown in this experiment. TGW has not been changed by breeding.

For further information on this aspect of the WGIN project contact Andrew Riche or Malcolm Hawkesford at Rothamsted (andrew.riche@rothamsted.ac.uk, malcolm.hawkesford@rothamsted.ac.uk).

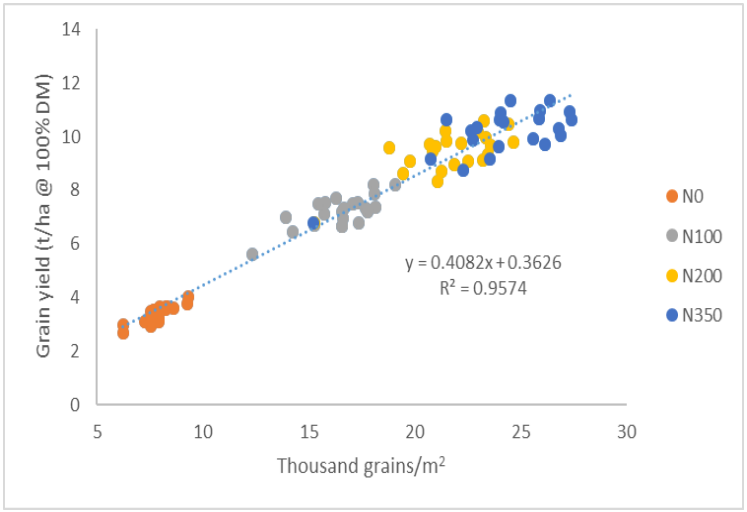

Figure 11: The effect of grain population on grain yield.

Resilience to Aphids (RRes)

Cereal aphids and the diseases they transmit have come to the forefront of the public's minds recently. This is because of the news of an impending neonicotioid insecticide ban in the UK causing concerns for the control of these regular cereal pests as well as Barley Yellow Dwarf Virus, which they transmit via their saliva into the wheat plant. Both of the main cereal aphid pests, *Rhopalosiphum padi* and *Sitobion avenae*, are known to be developing resistance to pyrethroid pesticides, which are the other main class of insecticides used to control them. This has made finding alternative control methods more pressing as there are no commercial wheat lines with resistance to either aphid species.

WGIN has developed mapping populations from *Triticum monococcum* lines showing partial resistance to both *R. padi* and *S. avenae*. These were chosen after extensive phenotyping of ~1000 different wheat varieties, ranging from elite wheat varieties through to diploid ancestors of wheat. These have now been screened through to the F_3 population, assessing both aphid development and aphid reproduction on the lines (**Figure 12**).

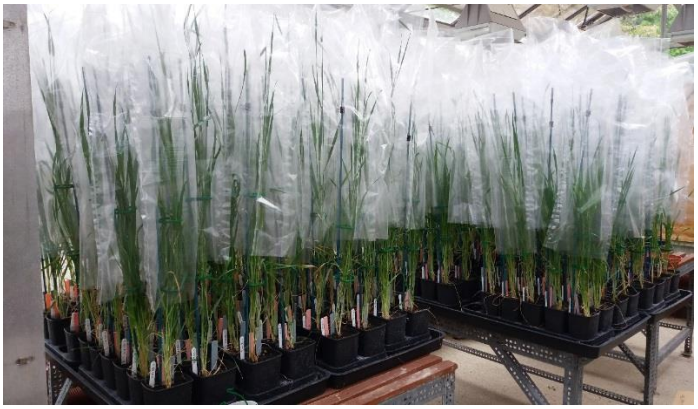

Figure 12: Mapping populations of *Triticum monococcum* used in screening for aphid resistance

Cross **MDR037 (susceptible) x MDR049 (resistant)** continues to show the best results, followed by cross MDR037 (susceptible) x MDR045 (resistant) and then MDR037 (susceptible) x MDR657 (resistant.). Overall the level of aphid resistance is slightly better for *S. avenae*, with a stronger reduction in development and nymph survival than *R. padi* (**Figures 13-15**).

All the tested lines in the populations have now been grown on to maturity and the seed harvested. Evaluation of the most promising F_4 population MDR037 x MDR049 will be continued in the BBSRC funded Designing Future Wheat Institute Strategic Programme, where it will be phenotyped and taken forward ultimately to the F_6 generation. Tissue samples have also been collected for QTL analysis.

The focus of aphid-wheat interactions in WGIN4 will move towards studying Barley Yellow Dwarf Virus transmission as well as tolerance or resistance to this aphid transmitted virus. With the imminent reduction in chemicals available to farmers, this is now becoming a high breeding priority and our change in direction reflects this need.

For further information on this aspect of the WGIN project contact Gia Aradottir at Rothamsted (gia.aradottir@rothamsted.ac.uk).

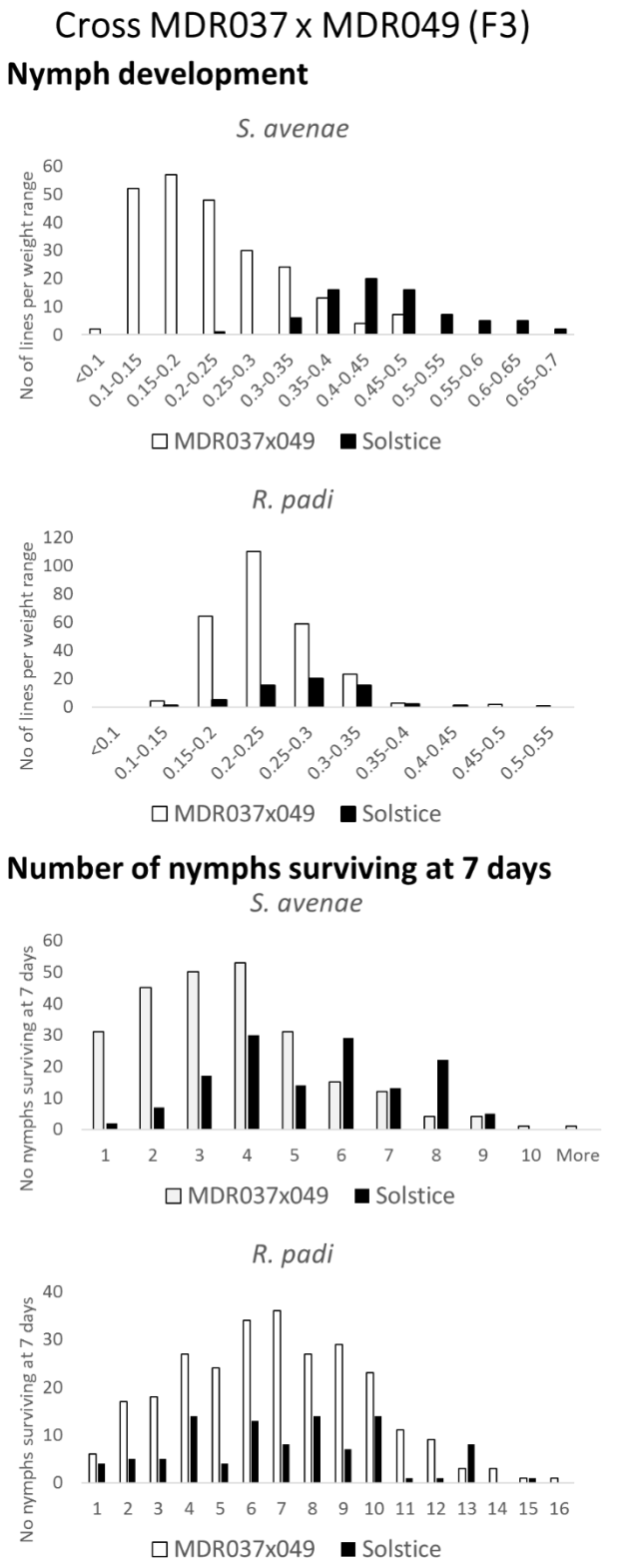

Figure 13: Nymph development and nymph survival on seven day old seedlings from the F_3 generation of cross **MDR037 x MDR049**. The aphid susceptible commercial variety Solstice was used as a control.

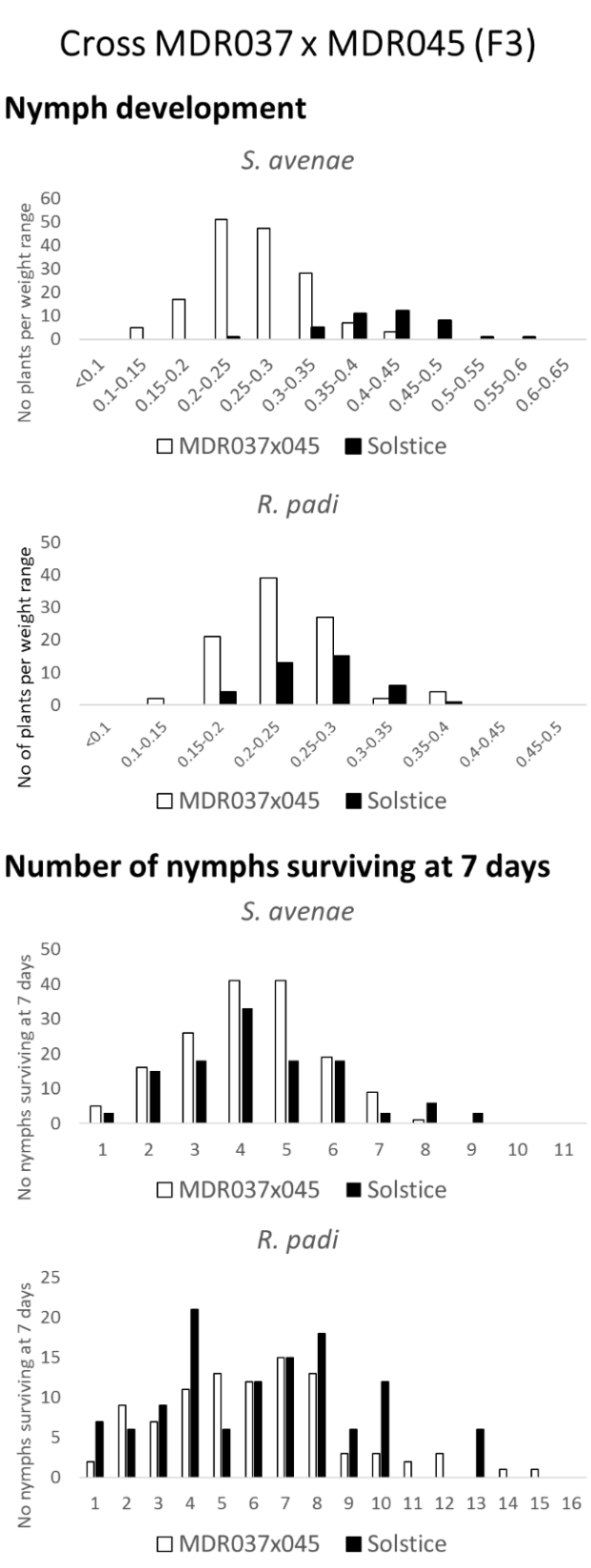

Figure 14: Nymph development and nymph survival on seven day old seedlings from the F3 generation of cross **MDR037 x MDR045**. The aphid susceptible commercial variety Solstice was used as a control.

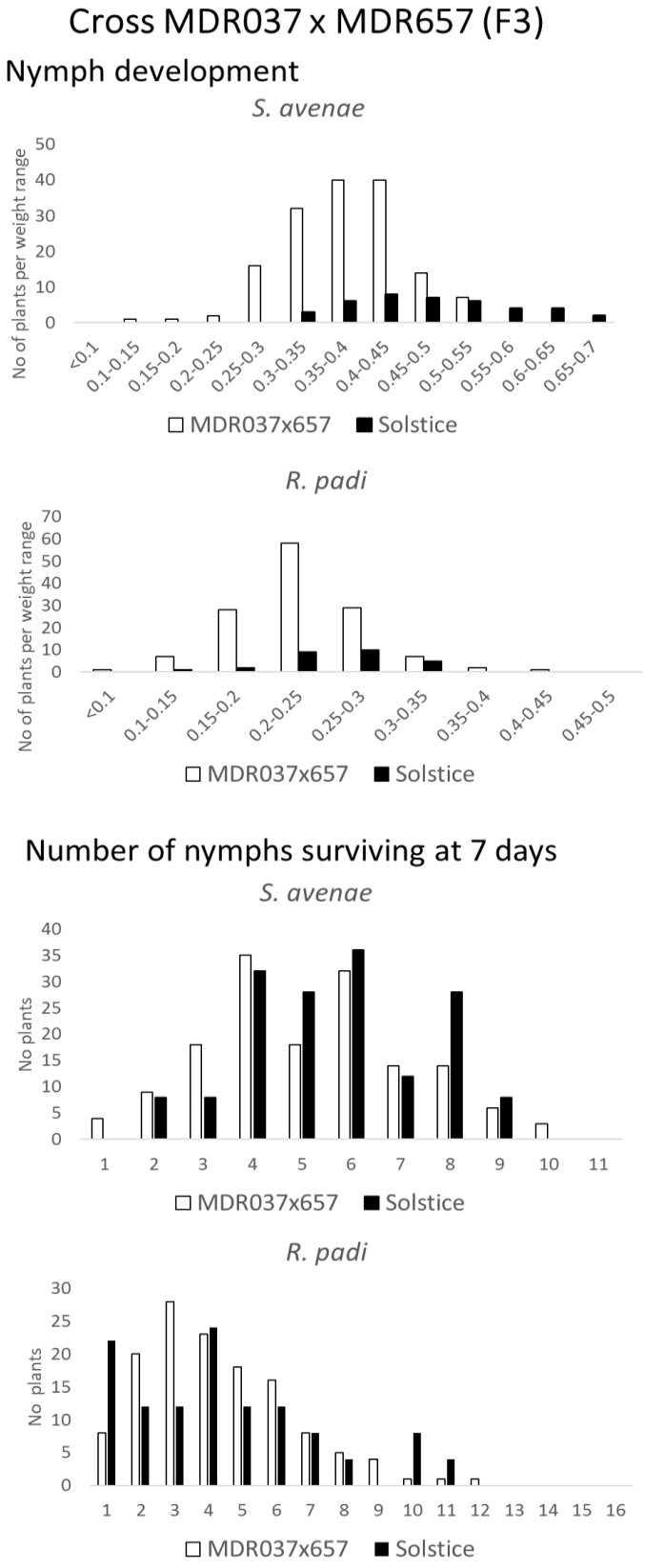

Figure 15: Nymph development and nymph survival on seven day old seedlings from the F3 generation of cross **MDR037 x MDR657**. The aphid susceptible commercial variety Solstice was used as a control**.**

Foliar Disease (RRes)

- **Exploiting resistance from hexaploid wheat landraces to foliar infecting fungal pathogens**

Foliar fungal pathogens are a major constraint on wheat productivity worldwide. In the UK and Europe *Zymoseptoria tritici* (Septoria leaf blotch), *Puccinia striiformis* (yellow rust), *Puccinia triticina* (brown rust) and *Blumeria graminis* (powdery mildew) are the major foliar pathogen threats to wheat crops. All four pathogens are difficult to control due to the development of fungicide resistance and strains able to overcome major host resistance genes within the pathogen populations. Identification and introduction of diverse disease resistance genes into new cultivars is important to improve yield stability and is a high priority for wheat breeders. Detailed information and characterisation of resistance sources is also required to better understand how the resistance genes function and interact and to inform resistance breeding programmes with the aim of producing durable cultivar resistance against multiple diseases. The Watkins landrace wheat collection (WGIN Newsletter February 2016) represents a potential novel source of resistance genes, already present in the hexaploid wheat background, which could be exploited for this purpose.

As reported in the previous WGIN Newsletter (June 2017) the foliar disease resistance of 10 Watkins genotypes (with low disease scores out of 740 Watkins genotypes screened in a heavily naturally infected single replicate field trial in 2008) is being examined in replicated field trials on the Rothamsted Farm. Six field trials across three field seasons have now been conducted and the average disease scores are shown in **Figure 16**. Yellow rust was present in all three field seasons with brown rust and septoria leaf blotch in 2015/16 and 2016/17 only. Overall Watkins genotypes 203 and 610 are the most promising for possessing multi-disease resistance with low disease scores against all three foliar pathogens (**Figure 16a**). However, Watkins 610 may be partially escaping disease through later leaf emergence. Septoria leaf blotch was at a generally low level across the trial sites but Watkins 203 and 610 both had lower levels of septoria than the susceptible modern hexaploid wheats Hereward and Paragon. A separate septoria specific trial protocol is currently being developed to examine their susceptibility to septoria in more detail in comparison to hexaploid wheats with known *Stb* resistance genes.

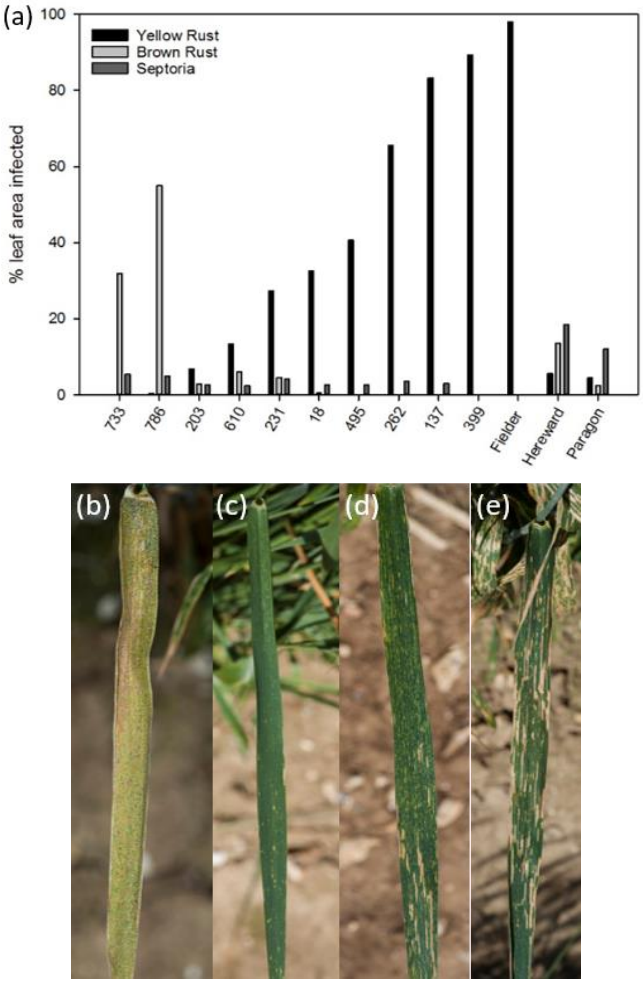

Figure 16: (a) Average disease scores across all three field seasons for 10 Watkins genotypes compared to the modern hexaploid wheat cultivars Fielder, Hereward and Paragon. Disease assessments carried out during flowering and/or grain filling. For some genotypes with very high levels of yellow rust infection (Fielder and Watkins 399) it was impossible to assess for septoria and brown rust. No significant powdery mildew infections developed in any field season, **(b)** the yellow rust susceptible control spring wheat cultivar Fielder with abundant yellow rust sporulation across the whole leaf surface, **(c)** the completely yellow rust resistant landrace Watkins 733 with no yellow rust sporulation and very little host response (i.e. >95% green leaf area) **(d)** the moderately to highly yellow rust resistant landrace Watkins 203 (which also shows resistance against brown rust and septoria) with some necrotic stripe formation but very little yellow rust sporulation, **(e)** the spring wheat cultivar Paragon with low levels of yellow rust sporulation but undesirable large necrotic striping host response across much of the leaf tissue

As reported in the previous WGIN newsletter, and now confirmed across all three field seasons, five of the 10 Watkins genotypes had moderate to strong resistance against yellow rust compared to the fully susceptible spring wheat cultivar Fielder (**Figure 16a-e**). Seedling tests under controlled environment conditions with three current yellow rust isolates will be carried out by NIAB in spring 2018 to compare to their adult plant field resistances. Watkins 733 and 786 were most resistant to yellow rust at the adult plant stage with no sporulation visible on Watkins 733 across any field season (**Figure 16c**). The modern hexaploid spring wheat Paragon was also highly resistant to yellow rust, with little yellow rust sporulation across all three field seasons. However, this genotype developed large necrotic stripes in response to yellow rust infection which is clearly an undesirable resistance phenotype (**Figure 16e**).

Field crossing was carried out in the first field season (2014/15) between the five moderately to highly yellow rust resistant genotypes (203, 231, 610, 733 and 786) and Fielder to generate mapping populations for the genetic characterisation. A marker screen on the Watkins genotypes for eleven known yellow and/or brown rust resistance genes suggests that their resistance is unlikely to be solely conferred by these major previously identified and commercially utilised rust resistance genes. In 2015/16 and 2016/17 six F_1 plants from each of the field crosses were grown in the field to study the inheritance of resistance. This revealed that yellow rust resistance was dominantly inherited in the case of Watkins 733 and Watkins 786 and semi-dominantly expressed in the F_1 for Watkins 203, 231 and 610 (**Figure 17**).

The genetic basis of resistance is now being further explored in the F_2, F_3 and backcross generations during the 2016/17 and 2017/18 field seasons. Preliminary analyses reveal that resistance to yellow rust in Watkins 733 may be conferred by two dominant unlinked loci (segregation ratio approaching 15 resistant to 1 susceptible plant in the F_2 generation). This will be confirmed by studying the F_3 and backcross generations and bulked segregant analysis carried out to identify the genomic location(s) involved. Segregation ratios for the Fielder x Watkins 203, 231 and 610 populations displayed more complex segregation ratios indicating that multiple loci may be involved. Future work will focus on mapping the locations of loci conferring resistance with the aim of identifying candidate genes and identifying closely linked genetic markers to select for the resistance in wheat breeding programmes.

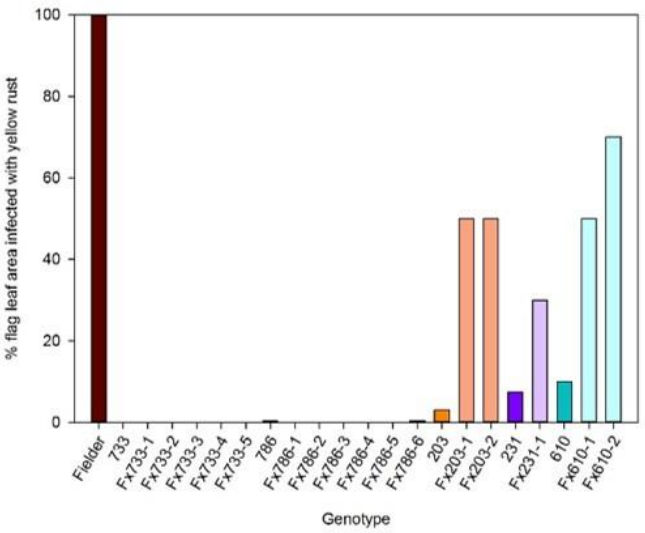

Figure 17: Yellow rust disease severity on individual F_1 plants from Watkins x Fielder crosses illustrating the inheritance of yellow rust resistance from the five Watkins accessions. Six F_1 grains from each cross were sown in the field with between 1 to 6 plants establishing and growing to maturity to be scored for rust infection

For further information on this aspect of the WGIN project contact Vanessa McMillan at Rothamsted (vanessa.mcmillan@rothamsted.ac.uk).

Introgression of *Triticum monococcum* Into Hexaploid Wheat – Light at the End of the Tunnel? (RRes)

Why is it important to be able to introgress *Triticum monococcum* into the hexaploid *Triticum aestivum* wheat? It appears that the continuing focus on breeding for yield increase over several decades has inadvertently resulted in a loss of resistance of most hexaploid commercial wheat varieties to pathogens and pests, resulting in potentially huge losses at harvest without the continuing and continual use of costly and time consuming pesticide applications.

Studies done in WGIN 1 & 2 established that a significant number of the genetically purified accessions of the diploid wheat *T. monococcum* are intrinsically resistant against a large number of commercially important pathogens, but it appeared that individual *Tm* germplasms

showed resistance against specific pests and pathogens. The *Tm* germplasm collection assembled at Rothamsted during WGIN1 thus contains many useful traits including resistance against **Take-all**, **aphids** and ***Septoria***, with the most resistant *Tm* germplasms being **MDR031**, **MDR049** and **MDR308**, respectively.

Previous attempts at crossing these three and other *Tm* cultivars with hexaploid wheat during WGIN1&2 had not resulted in the production of any viable grain at the F_1BC_1 stage. While using the ***Ph-1*** (pairing locus) mutant of Paragon (Graham Moores, JIC) as the female parent showed initially promising results (as reported in a previous WGIN newsletter) with over 500 F_1 grains generated and harvested, it was not possible to generate self-fertile plants from these grains. In fact, none of the grains tested (around 10%) could be germinated. Embryo rescue was employed for 25 grains but eventually **only 1** grain grew into an apparently healthy plant, which unfortunately was **both male and female sterile**. Interestingly, this lone F_1 plant did have (sterile) pollen inside the anthers.

As reported here, the next strategy employed **tetraploid wheat** as a bridging species. The crossing strategy is shown in **Figure 18**. Potential **F_1** grains would be **A^mAB triploid,** but also most likely **male sterile**. From the literature, it appears that the success rate for female fertility is around 0.5%, suggesting the necessity for a large number of pollinations with the fertile hexaploid pollen. The resulting '**F_1 complex**' is thought to be **pentaploid**, missing one Paragon D genome and substituting one Paragon A genome for one *Tm* **A^m** genome or segments of A^m chromosomes within the Paragon A genome (not shown). Subsequent backcrosses (BC_2F_1 to BC_nF_1) would eventually generate the desired hexaploid hybrid **A^mABBDD.** The results of the first round of crossing *T. monococcum* to *T. durum* is shown below (**Table 4**).

Table 4: 1st round of hybrid crosses between tetraploid *Triticum durum* and diploid *Triticum monococcum* (MDRs)

cross	Grains (F_1)	Ears
Kronos x MDR031	7	7
Kronos x MDR049	4	3
Kronos x MDR308	12	6
Hoh501 x MDR031	8	4
Hoh501 x MDR049	3	3
Hoh501 x MDR308	0	0

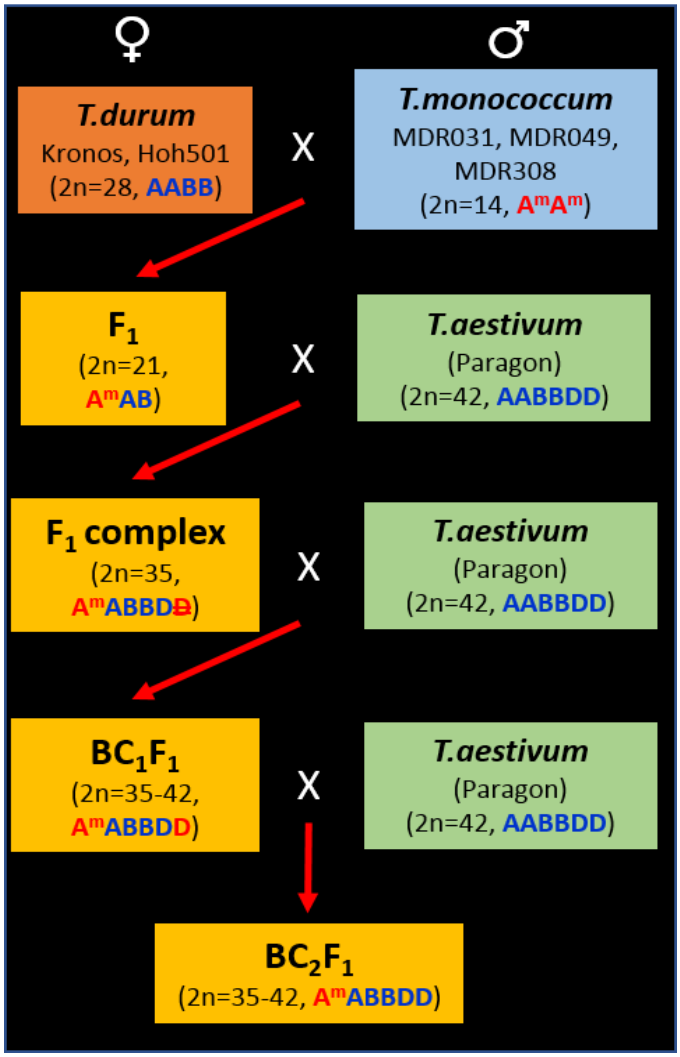

Figure 18: Crossing strategy for Introgression of *T. monococcum* into Hexaploid Wheat. Please note that all germplasms shown on the left are used as the females, to be pollinated with the fertile anthers of the male germplasms on the right (as indicated by the gender signs).

Although the total number of grains obtained was small, reflected in the small number of ears that could be pollinated, there were enough grains to continue with this crossing strategy. All grains were **pre-germinated** with a 100% success rate - notably unlike the Paragon *ph-1* x *Tm* crosses (see above) - and subsequently put into vernalisation for either **four** weeks (Kx031, Kx308) or **eight** weeks (Kx049, Hx031, Hx049). As apparently these triploid F_1 plants quite often grow only with the primary or very few additional tillers, which would make a large number of pollinations impossible, it was suggested to grow all hybrid plants under intermediary glasshouse conditions (15°C,

12h day length, 10°C o/n) for three weeks (Richard Horsnell, NIAB), followed by normal conditions thereafter.

This approach resulted in between 12 to 16 tillers for all F_1 hybrids. While we decided not to use any of the precious F_1 grains as a control to grow under normal glasshouse conditions, this was done for Paragon. Plants first grown under intermediate conditions had 12 tillers on average (±1.5), while plants grown under normal conditions only had 9 tillers on average (±2), suggesting that this intermediary approach could be used more widely if the number of tillers is of concern.

All backcrosses involving *T. aestivum* used 'good' Paragon, kindly provided by Simon Orford (JIC). The results of the F_1 hybrids x Paragon crosses are shown **in Table 5**.

Table 5: No of F_1 stigmas pollinated with Paragon and 'F_1 complex' grains obtained

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

The success rate lay between 0.47% - 0.83% and thus around the expected success rate of 0.5% (see above). These results are very promising, at least for Take-All resistance (MDR031). Furthermore, the subsequent crosses to generate BC_1F_1 and BC_2F_1 should generate a significant number of grains. It appears that of the three *Triticum monococcum* accessions used, only MDR049 is somewhat recalcitrant because with all things being equal, statistically one would have expected 1 grain for Kronos and 2-3 for Hoh501.

Generally, the hybrid plants exhibited a lot of *Tm* characteristics, most notably height and hairiness of nodes. **Figure 19** shows a comparison of a triploid Hoh501xMDR031 F_1 and Hoh501 parent plant (a) and ears ((b), (c)) as well as one of the presumably pentaploid 'F_1 complex' grains obtained (d). Very noticeable is the height increase of the F_1 hybrids (up to twice as high as the tetraploid parent), conferred by the *Tm* portion of the triploid F_1 genome. Please note that **none** of the F_1 hybrid plants had any pollen inside the anthers which nevertheless still extruded like their pollen-filled

Figure 19: a) Comparison of heights of triploid F_1 plant (left) and Hoh501 parent **b)** selfed Hoh501 grain-filled ear **c)** 'selfed' Hoh501 x MDR031 F_1 sterile ear (empty) **d)** ripening 'F_1 complex' grain. Note extruded anthers in c) and d) which never contained any pollen**.**

counterparts (**Fig19d**). Interestingly, this is different to the earlier attempt using *Tm* and the Paragon *ph*-1 mutant (see above). Because of the absence of pollen, self-fertilisation was impossible and emasculation was only carried out for the first few ears (of 125 total ears pollinated).

So far, this promising new crossing scheme appears to have only worked well for the MDR031 introgression. Therefore, a second round of introgression (stage1) has just been completed, concentrating on MDR049 and MDR308. While some ears remain to be scored, the results so far are shown in **Table 6**. This second round of F_1 hybrid generation has been very successful, which, assuming a similar success rate for the F_1 hybrid crosses to Paragon as shown above, should lead also to the introgression of MDR049 and MDR308 into hexaploid wheat. So, it appears there might indeed be light at the end of the tunnel.

Table 6: 2nd round of crosses between tetraploid *Triticum durum* and diploid *Triticum monococcum*

cross	Grains (F_1)
Kronos x MDR049	0
Hoh501 x MDR049	13
Kronos x MDR308	11
Hoh501 x MDR308	79

For further information on this aspect of the WGIN project contact Michael Hammond-Kosack at Rothamsted (michael.hammond-kosack@rothamsted.ac.uk).

The WGIN Promotome Capture Experiment (RRes)

- **Initial Outcomes and Analyses**

Please note that the WGIN Promotome Capture experiment and analyses relied heavily on the prepublication release and use of the **IWGSC Chinese Spring refseq1.0 sequences**. (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies).

This experiment has been very successful and has achieved the goals set, as shown below. This success was without doubt in a large part due to the very high technical skills of the subcontractor **MYcroarray** (since renamed **Arbor Biosciences**) for carrying out the MYbaits capture but also their very thorough customer care and solid advice in designing the MYbaits probes. Particularly their insistent suggestion (Dr. Jacob Enk) to use the highest stringency filtration of MYbaits probes and discard any baits that could cross-hybridise to homoeologous promoter sequences ensured the success of this project.

One of the main objectives of this project was to capture (and sequence) individual homoeologous promoters for a total of **1355 genes** (received from the trait co-ordinators) within **10 trait categories** chosen by the WGIN3 Management Team (**Figure 20**) in **96 wheat cultivars**, which included a large number of commercial wheat varieties as well as several used in scientific research (**Table 7**).

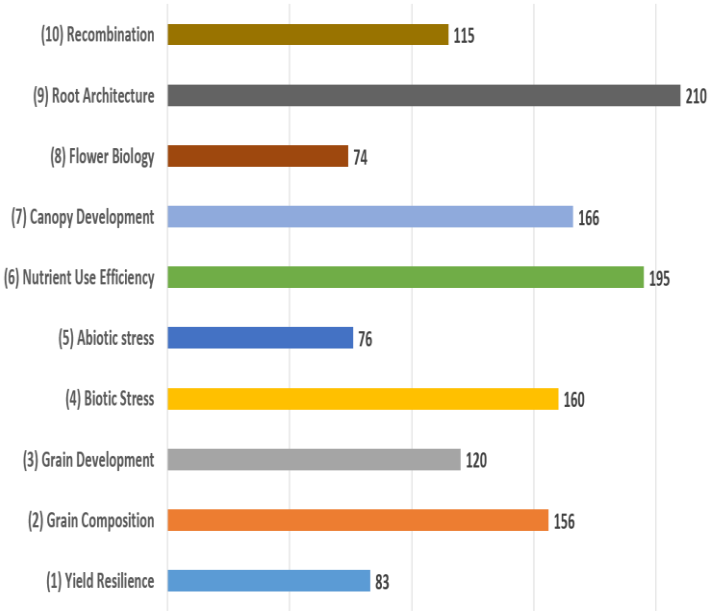

Figure 20: The **10 Trait categories** for the WGIN Promotome Capture project and numbers of **Gene IDs** received from the **Trait Co-ordinators**. Please see page 77-78 of the online presentation of the WGIN Management Meeting October 6th 2017 for details of the trait coordinators and 96 wheat cultivars included in this project.

Two of the diploid and tetraploid cultivars (highlighted in **Table 7**) were included in the experiment to be able to determine the **homoeologue-specific capture** as follows: Kronos contains the A & B genome of hexaploid wheat while *Aegilops tauschii* (ENT-228) contains **only** the D genome. Thus, any capture using A or B homoeologue MYbaits can be considered **specific** for these homoeologues when good sequencing coverage using

these probes is **only observed** for Kronos **but NOT** for *Aegilops tauschii*. The reverse is true for any D homoeologue MYbaits - sequencing coverage would only be expected for *A.tauschii* **but NOT** for Kronos. This is summarised in **Table 8**.

Table 7: The 96 wheat varieties grouped by ploidy level and use. Please use link above for details of individual cultivars

ploidy	use	no. of cultivars	details
diploid	academic	10	*T.monococcum* [A^mA^m](8x), ***A. tauschii* [DD]**, *A. speltoides* [SS]
tetraploid	academic/ commercial	2	**Kronos [AABB]**, *A. peregrina var* [SSUU]
hexaploid	academic	15	Watkins landraces
hexaploid	mostly commercial	69	

The other diploid and tetraploid species do not allow such strict reasoning, because their genomes are only **related** to either the A, B or AB genome of hexaploid wheat, namely *T.monococcum* (A^m A^m), A. *speltoides* [UU ~ BB] and *A.peregrina var* [SSUU ~ AABB], respectively. But coverage predominantly for the A, B and AB homoeologues would still be expected, respectively.

Table 8: MYbaits are specific to each homoeologue **only** if the below coverage pattern is observed. KR = Kronos (AABB), ENT = *A.tauschii* (D)

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

At this time, the initial analysis has been completed by MHK for **yield resilience** (trait 1), **grain composition** (trait 2) and **biotic stress** (trait 4) with regards to homoeologue specificity, sequencing coverage (promoter length, 5' UTR, Exons, Introns) and SNP pattern similarities. But as explained above, unfortunately NO specific details can be reported here at present.

As discussed above, the expected and hoped for presence (AB) and absence (D) of captured promoters for **Kronos** is reversed as expected for ***Ae. tauschii*** and this demonstrates that it has been possible to **specifically** capture individual homoeologue promoters for the vast majority of genes (**Table 9**).

Also for ***T. monococcum***, most of the promoters were only captured with the A homoeologue specific baits (92.3%) and for ***Ae. speltoides*** a narrow majority for the B homoeologues was observed (55.1%). **Only** for ***Ae. peregrina variabilis,*** contrary to expectations, **more** full length promoters were captured for the D genome (**Table 9)**. In this regard, it is worth mentioning that *Ae. peregrina* has consistently the most divergent sequence (compared to IWGSC Chinese Spring refseq1.0) of all the 95 (out of 96) cultivars analysed (data not shown). Please note that Promotome Capture failed for one cultivar, Watkins 239.

Table 9: Homoeologue promoter coverage (%) for the diploid and tetraploid cultivars of the WGIN Promotome capture

cultivar	homoeologue		
	A	B	D
Kronos	**50.2**	**48.7**	1.1
Ae. tauschii	0.8	0	**99.2**
T. monococcum	**92.3**	2.9	4.8
Ae. speltoides	17.4	**55.1**	27.5
Ae. peregrina	24.3	27.1	**48.6**

The probes (MYbaits) for the WGIN Promotome Capture were designed to capture **1700bp of promoter and 5'UTR** up to the ATG start codon. As we opted for the high stringency MYbaits design (see above), MYbaits coverage of the 1700bp sequences was reduced:

- 71% of promoters covered by ≥50%
- 29% of promoters covered by ≤50% of MYbaits.

How this translates into the actual lengths of [promoter+5'UTR] captured for traits 1, 2 and 4 is shown in **Table 10**.

As can be seen, the vast majority of genes have over 1000bp of sequence captured, with ≥1700bp capture for 59.5% of trait 1 genes, 75.5% for trait 2 genes and 53.4% for trait 4 genes (**Table 10**). This is a very satisfactory

outcome because it combines rigid homoeologue specificity with good to very good promoter lengths.

Table 10: [promoters&5'UTRs] captured, grouped by lengths (shown as % of trait genes)

	>1000bp	>1500bp	>1700bp
Trait 1	95.9%	71.6%	59.5%
Trait 2	91.4%	71.6%	75.5%
Trait 4	85.3%	66.9%	53.4%

More data will be available in due course, as soon as the restrictions regarding the display, sharing, or publishing in any format of data using the IWGSCrefseq1.0 are removed.

For further information on this aspect of the WGIN project contact Michael Hammond-Kosack at Rothamsted (michael.hammond-kosack@rothamsted.ac.uk).

Section 3 Events

- The **15th WGIN Stakeholders' Meeting** was held jointly with the BBSRC Designing Future Wheat project @ Rothamsted Research on **Thursday November 30th 2017**. Information and video interviews from this event can be accessed at https://www.rothamsted.ac.uk/news/combine-harvesters

- UK wheat scientists will visit **Kazakhstan from May 26th to June 2nd 2018** for a BBSRC-supported bilateral workshop on wheat genetic improvement with scientists from Kazakhstan and neighbouring countries (Tajikistan, Uzbekistan and Kyrgyzstan). All of these Central Asian countries grow wheat for their own consumption, but Kazakhstan is also eighth in the list of global wheat exporters. However, yields are constrained by high temperatures and low water availability.

  The fourteen UK delegates, which comprises scientists from Rothamsted Research, John Innes Centre, NIAB, and the Universities of Nottingham, Bristol and Lancaster, will visit the Kazakh Cereals Research Institute near Astana and the Kazakh Cereals Research Institute and Institute of Plant Biology and Biotechnology near Almaty.

  In addition, formal workshop sessions and informal discussions will facilitate the exchange of information and germplasm and the development of joint research programmes and grant applications.

- A one-day Promotome Workshop (a strictly closed meeting for WGIN wheat breeders and trait-coordinators) is being organised to be held at RRes, on **June 6th 2018**.

Section 4 News

The WGIN project has been further extended by Defra. The £1.7 million of new funding provides for a 5-year programme that extends WGIN, founded in 2003, to at least 2023. The new contract, due to be signed in May 2018, dovetails with the running down of WGIN 3. For the first time, the network's new contract includes a formal alliance with the Agriculture & Horticulture Development Board (AHDB) with the aim of strengthening direct links to farmers.

Section 5 Publications 2016 to 2018

2018

- Saintenac, C., Lee, W-S., Cambon, F., Rudd, J.J., King, R., Marande, W., Hélène Bergès, Phillips, A.L., Uauy, C., Hammond-Kosack, K.E., Langin, T., and Kanyuka, K. (2018) An evolutionary conserved pattern-recognition receptor like protein controls gene-for-gene resistance to a fungal pathogen in wheat. Nature Genetics **50**,368–374, doi:10.1038/s41588-018-0051-x
- McMillan, V.E., Canning, G., Moughan, J. R.P. White, J.R.P., Gutteridge, R.J. and Hammond-Kosack, K.E. (2018) Exploring the resilience of wheat crops grown in short rotations through minimising the build-up of an important soil-borne fungal pathogen. Nature Scientific Reports (in press May)
- Osborne, S.-J., McMillan, V., White R. and Hammond-Kosack, K. E. (2018) Elite UK winter wheat cultivars differ in their ability to support the colonisation of beneficial root-infecting fungi Phialophora. Journal of Experimental Botany (April, doi: 10.1093/jxb/ery136.)

2017

- Acevedo-Garcia, J., Spencer, D., Thieron, H., Reinstädler, A., Hammond-Kosack, K.E**.,** Phillips A.L. and Panstruga, R. (2017) *mlo*-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach. Plant Biotechnology Journal 15, 367-378

2016

- Gardiner, L-J, Bansept-Basler, P., Olohan, L., Joynson, R., Brenchley, R., Hall, N., O'Sullivan, D.M. and Hall, A. (2016) Mapping-by-sequencing in complex polyploid genomes using genic sequence capture: a case study to map yellow rust resistance in hexaploid wheat. The Plant Journal 87, 403-419. No author funded by WGIN. This study used the A x C mapping population resource.
- Harper, A., Trick, M., He, Z., H., Clissold, L., Fellgett, A., Griffiths, S., Bancroft, I. (2016) Genome distribution of differential homoeologue contributions to leaf gene expression in bread wheat Plant Biotechnology Journal 14(5):1207-14. doi: 10.1111/pbi.12486
- Jones H., Lukac M., Brak B., Martinez-Eixarch M., Alhomedi A., Gooding M., Wingen L., Griffiths S. (2016) Photoperiod sensitivity affects flowering duration in wheat The Journal of Agricultural Science FirstView 1-12.
- Kowalski A., Gooding M., Ferrante A., Slafer G., Orford S., Gasperini D., Griffiths S. (2016) Agronomic assessment of the wheat semi-dwarfing gene *Rht8* in contrasting nitrogen treatments and water regimes Field Crops Research 191, 150-160

For further information on any aspect of the WGIN project please go to www.wgin.org.uk or contact us at wgin.defra@rothamsted.ac.uk.

The contributors to this newsletter were: Kim Hammond-Kosack, Malcolm Hawkesford, Andrew Riche, Peter Shewry, Vanessa McMillan, Gia Aradottir, Michael Hammond-Kosack (**RRes**); Clare Lister and Simon Griffiths (**JIC**).

HAVE YOU HEARD ABOUT THE PARAGON LIBRARY?

WGIN has been part of an informal consortium developing NILs in the genetic background of the UK spring wheat **Paragon**. The collection, known as the **Paragon Library**, was developed at **JIC** and currently consists of around 350 lines.

The project involves crossing different combinations of genes, QTLs and mutations into the common background of Paragon and then studying the phenotypic effects. This uniform genetic background will provide a unique insight into the potential value of these genetic effects for UK breeding and agriculture.
Most of the effects were discovered in work funded by DEFRA, the BBSRC and AHDB and represent hundreds of person-years of research.

Most of the Paragon Library has already been trialled in 1 m and 6 m plots for the duration of WGIN. Phenotypic data from these trials (phenotypes underlying grain yield and crop adaptation) should become available on the WGIN website from spring 2018.

The Paragon Library will be genotyped on the Axiom 35k Breeders' Array shortly and seed from the genotyped plants will be used to generate the resource for distribution.

NILs are available for multiple alleles of:
Rht-D1, Rht-B1, Rht8, Ppd-B1 Ppd-D1, Lr19, 1BL.1RS and *7B* (yield), 10 Heading Date QTL, *Vrn1, Vrn3*, grain size (5A, 7A), and selected WGIN mutants, such as EMS.

Clare Lister and Simon Griffiths