Department for Environment Food & Rural Affairs

WGIN Stakeholders' Newsletter

Wheat Genetic Improvement Network

Welcome to WGIN4 !

Defra Wheat Genetic Improvement Network (WGIN4): Improving the resilience of the wheat crop through 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 (1.86 m hectares) and is more valuable than any other arable crop in the UK. The Wheat Genetic Improvement Network (WGIN) started in 2003, is funded by the Department for Environment, Food & Rural Affairs (defra). The overall aim of WGIN is to generate pre-breeding material carrying novel traits for the UK breeding companies and to deliver accessible technologies and new knowledge, 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 **4th phase of WGIN (WGIN4)**, which is funded for 5 years, started in February 2018 and is entitled '**Improving the resilience of the wheat crop through genetics and targeted traits analysis**'. This project consisted of four work packages (WPs) (**Figure 1**). WP1 focusses on further enhancing the networking and communication activities. Two new activities are included in WP1. Firstly, via the newly formed project management group (PMG) and the research advisory group (RAG), we are in process of aligning WGIN to the other three defra funded GINs, namely PCGIN for pulse crops, OREGIN for oilseed rape and VeGIN for field and leafy vegetables (http://www.wgin.org.uk/about/GINs.php). Secondly, AHDB has become involved to a far greater extent in disseminating the GINs activities and our key research findings to UK based farmers, growers and crop advisors. This is being done through the AHDB's strategic farm and monitor farm events programme. AHDB is also helping to promote the annual GINs stakeholder events more widely.

Within WGIN4 the three inter-connected research work packages (WP2, WP3 and WP4) (**Figure 1**) remain the same as in WGIN3. They are focussed on exploring a range of previous and newly nominated high priority traits for the

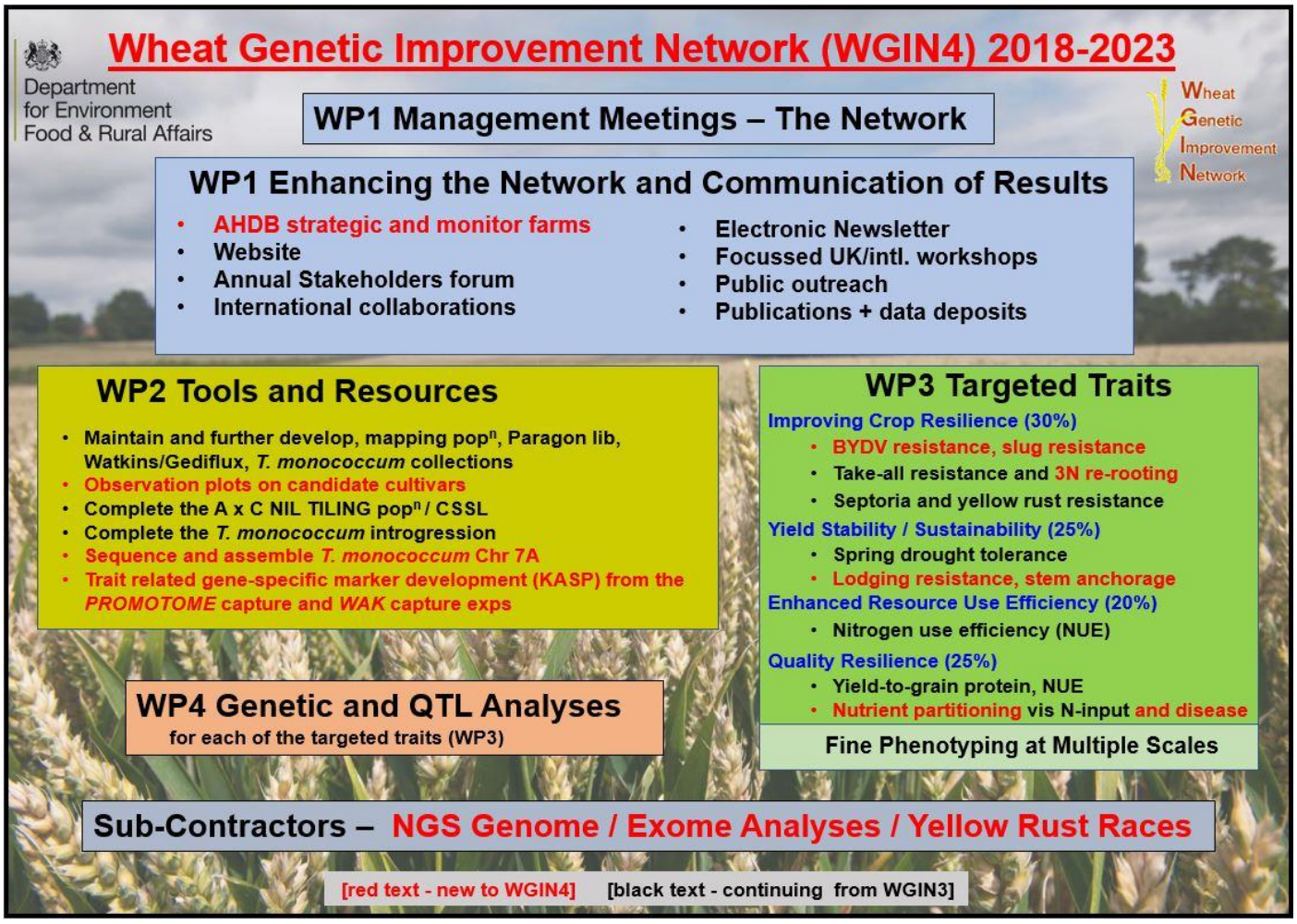


Figure 1 The organisational schematic of WGIN4. All red text highlights new additions compared to WGIN3

UK wheat crop, followed by detailed genetic and quantitative trait loci (QTL) analyses (WP3 and WP4), maintaining and developing new genetic resources for the UK research community (WP2), and testing new tools based on next generation sequencing technologies for their applicability to wheat research (WP4). In WGIN4, 70% of the research effort is focussed on traits analyses. The split of this research effort between the four overarching traits is resilience (30%), yield stability / sustainability (25%), quality resilience (25%) and resource efficiency (20%).

The funded partners in WGIN4 are the **John Innes Centre** (JIC), **Rothamsted Research** (RRes) and five sub-contractors, the **Bristol Genomics Facility**, based within the University of Bristol, **Affymetrix** (providers of the Axiom®35K breeders' array), the company **Arbor Biosciences** (previously MYcroarray), based in Michigan, USA, for high throughput sequence capture experiments for multiple genes present in multiple wheat varieties, **Dovetail Genomics**, San Diego, USA, for de novo sequencing and assembly of an isolated wheat chromosome and the **National Institute of Agricultural Botany** (NIAB) for screening new sources of resistance with specific races of yellow rust. The project also has an overseas academic collaborator **Professor Jaroslav Dolezel**, Centre of Plant Structural and Functional Genomics, Olomouc-Holice (Czech Republic).

Section 2 Research Updates

WGIN4 at the JIC

With the funding from defra for WGIN4, new projects will be initiated as well as continuing delivery of core WGIN activities, including development of the **next generation of Avalon x Cadenza** resources such as the CSSL lines (see below) and **availability of the Paragon Library**.

- **Yield Stability/Sustainability Traits**

Wheat growing conditions are increasingly under pressure from variable climate conditions, in particular volatile and rising temperatures and unpredictable rainfall. The requirement for high yield, with consistency between locations and years is therefore an increasingly important target. There is strong evidence that improved drought tolerance is a major route to increased yield stability and resilience to climate change. Spring drought is now a common element of UK climate and presents a unique target for trait improvement in UK wheat as this can severely affect grain number. At JIC, we are particularly concentrating on yield stability traits in WGIN4 and this includes continuing our work on spring drought from WGIN3 but also examining lodging and stem (essentially root) anchorage. There are several reasons for extending this research. Extreme weather conditions, particularly involving heavy rain and strong winds or water stress, will inevitably lead to a rise in lodging and crop loss, so identifying lines which are lodging resistant is an important target. New tools that allow us to breed for lodging resistance could make a huge impact on yield stability. In addition, drought is likely to affect root growth and hence stem anchorage. It is possible that drought could increase anchorage as roots grow longer to search for water. Conversely, root systems may be shorter as roots are unable to grow through dry baked soil. To this end we have assembled a 'Drought, Anchorage and Lodging Panel' (**DALP**) in which the lines may potentially have drought tolerance / sensitivity and/or lodging resistance / sensitivity. These lines come from a multitude of sources including derived materials generated in WGIN, the DFW Breeders' Toolkits (2017 and 2018), UK varieties from breeders, CIMMYT varieties from the SAWYN panel (Semi-arid Wheat Yield Trial**)**, the Rothamsted WGIN Diversity Panel, the Watkins collection (obtained in the 1930s from Mediterranean countries), promising RILs from the Paragon x Garcia drought-tolerant population (DT), the parents of existing mapping populations and parents of crosses between RL lines, and even a tall oat! (see **Table 1**). Note - there is a small amount of duplication of the same material from different sources.

The DALP has been drilled as single 1 m plots for multiplication and initial observations in autumn 2018 and will also be used to continue developing an anchorage strength test (**and any suggestions from readers on how to do this are very welcome!**).

- **Dissecting UK drought tolerance in Paragon x Garcia**

UK wheat is susceptible to spring drought at the start of stem extension (stage 31) when *grain number* begins to be determined and is likely to cause yield reduction. In the last 10 years, drought in East Anglia has occurred seven times during April, coinciding with this vulnerable period. Drought during *grain filling* (mid June-July) will also affect yield. For the last three years (2016-2018) 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). 177 Paragon x Garcia (PxG) RILs plus nine lines from the Paragon Library (PL), with Paragon and Garcia controls and Soisson as the marker, totalling 200 lines, were selected for the Drought Trials.

Table 1 Details of lines included in the Drought, Anchorage and Lodging Panel (DALP)

Possible Drought and/or Lodging lines	RL crosses	Parent of other crosses	CIMMYT	RRes
Alchemy				
Atilla				
Avalon				
Baj				
Barrel				
Becard Kachu				
Beluga				
Borlaug 100				
Cadenza				
Charger				
Chinese Spring				
CIMCOG 47				
CIMCOG 49				
Claire				
Conqueror				
Cordiale				
Cordiale 3N (Rec 5-1)				
Costello				
Cougar				
Crusoe				
Denman				
DFW BTK H17 (x3)				
DFW BTK H18 (x3)				
Einstein				
Fiorello				
Freiston				
Gallant				
Garcia				
Glasgow				
Grafton				
Graham				
Hereward				
Horatio				
Hylux				
Icon				

Invicta				
Istabraq				
JB Diego				
KWS Croft				
KWS Gator				
KWS Kielder				
KWS Santiago				
KWS Silverstone				
KWS Siskin				
KWS Sterling				
KWS Zyatt				
LG Motown				
LG Skyscraper				
LG Sundance				
Lr19				
Malacca				
Maris Widgeon				
Mascani - OAT!				
MISR1				
Pamyati Azieva				
Panorama				
Paragon				
Paragon EMS semi dwarves (x5)				
Paragon Rht D1 x B1				
Paragon RhtB1				
Paragon RhtB1 x Rht8				
Paragon RhtD1				
Paragon RhtD1 x Rht8				
Pastor				
Pfau				
Pomerelle				
Reflection				
Revelation				
RGT Illustrious				
Rht8				
Riband				
Robigus				
Savello				
Scout				
Siskin				
Skyfall				
Soisson				
Sokoll				
Solstice				
Spark				
Super 152				
Synth Type				
Treasure				
Watkins 110				
Watkins Indian dwarfs W126				
Watkins Indian dwarfs W127				
Waxwing				
Weebill				
Wyalkatchem				
Xi19				
Zyat				
Paragon x Garcia RILs (15)				

The trials consist of two randomised reps each of all lines in Not Irrigated (NI) and Irrigated (IR) 6 m^2 plots. The climate conditions have been variable during spring and summer in these years (see **Figure 2**) with April drought only occurring in the spring 2017, and a severe summer drought in 2018. This has led us to drilling the Paragon x Garcia Drought Trial for a fourth time in the hope of a dry April 2019!

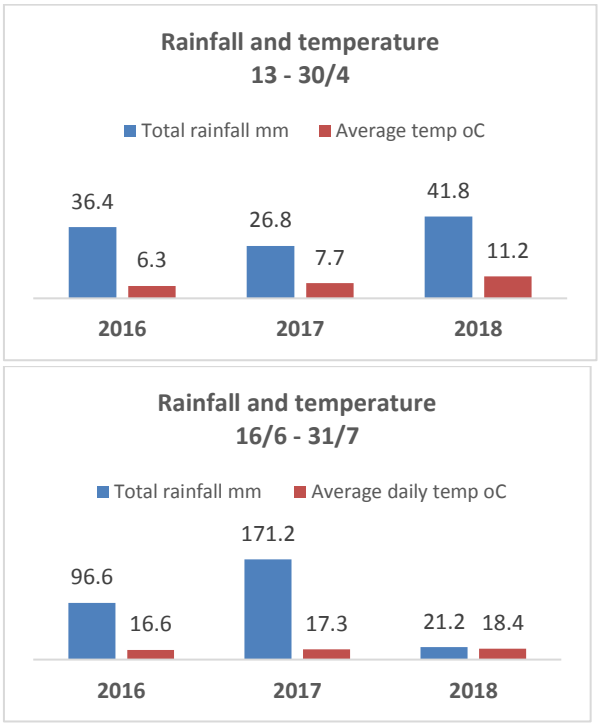


Figure 2 Total rainfall and average temperatures from mid-April to the end of April and mid-June to the end of July for 2016 – 2018 at Church Farm near the JIC, Norwich

Despite the less than ideal conditions for this experiment in 2016 and 2018 we were still able to generate some consistent results over the three years and identify chromosome regions of interest. Yield traits were scored and this phenotype data was put together with genotype data for QTL mapping. **Table 2** shows the Yield QTLs identified in the 2016, 2017 and 2018 trials. The most notable QTLs are Q1A-1, Q2B-1 and Q2B-2, Q4D-1, Q5B-1 and Q7A-1. Based on the 2016 and 2017 results two of these QTLs (Q1A-1 and Q2B-2) have already been included in a NIL production programme and we would hope for inclusion of the others in the future.

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

WGIN has successfully promoted the AxC doubled haploid population (DH) as the UK reference and it is now the most densely mapped wheat population in the world. We have been developing a unique resource using these lines. More details of the concept behind this experiment, the experimental procedure and preliminary results were presented in the 2017 and 2018 WGIN newsletter http://www.wgin.org.uk/ stakeholders/ newsletters.php.

Table 2 Yield QTLs from the Paragon x Garcia Drought Trials 2016-2018

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

We have selected 94 NILs, from the Cadenza and Avalon recurrent backgrounds, with QTL for heading (EM)), height (HT) and yield (YLD), (**Table 3**). The selected lines were backcrossed to the recurrent parent (Avalon or Cadenza) and self-fertilised. DNA from the selected NILs was genotyped at the Bristol Genomics Facility on the Breeders' 35K Axiom® array**.** Maps showing the extent of the QTL and random background regions in each NIL were produced, using the genotyping data and the IWGSC RefSeq v1.0 (genomic sequence of Chinese Spring) (see **Figure 3**). Where possible, KASP markers were designed to pick up all introgressed regions across the genome. For example, most of the introgressed regions in CSSL47, on chromosomes 3A, 7A and 7D, can be identified with markers (yellow stars) as shown in **Figure 3**.

Table 3 Selected NILs for CSSL generation: NILs in an Avalon background with a QTL region from Cadenza, and *vice versa***.**

QTL from Cadenza	in Avalon background
1B EM	x 5
1D EM	x 3
2A HT	x 1
2D HT	x 5
2D YLD	x 3
3A HT	x 5
3B HT	x 4
5A YLD	x 4
6A HT	x 5
6B HT	x 5
7B YLD	x 1
7D YLD	x 3

QTL from Avalon	in Cadenza background
1B EM	x 5
1D EM	x 5
2A HT	x 5
2D HT	x 6
3A HT	x 6
3B HT	x 5
3B YLD	x 5
6A HT	x 5
6B EM & HT	x 5

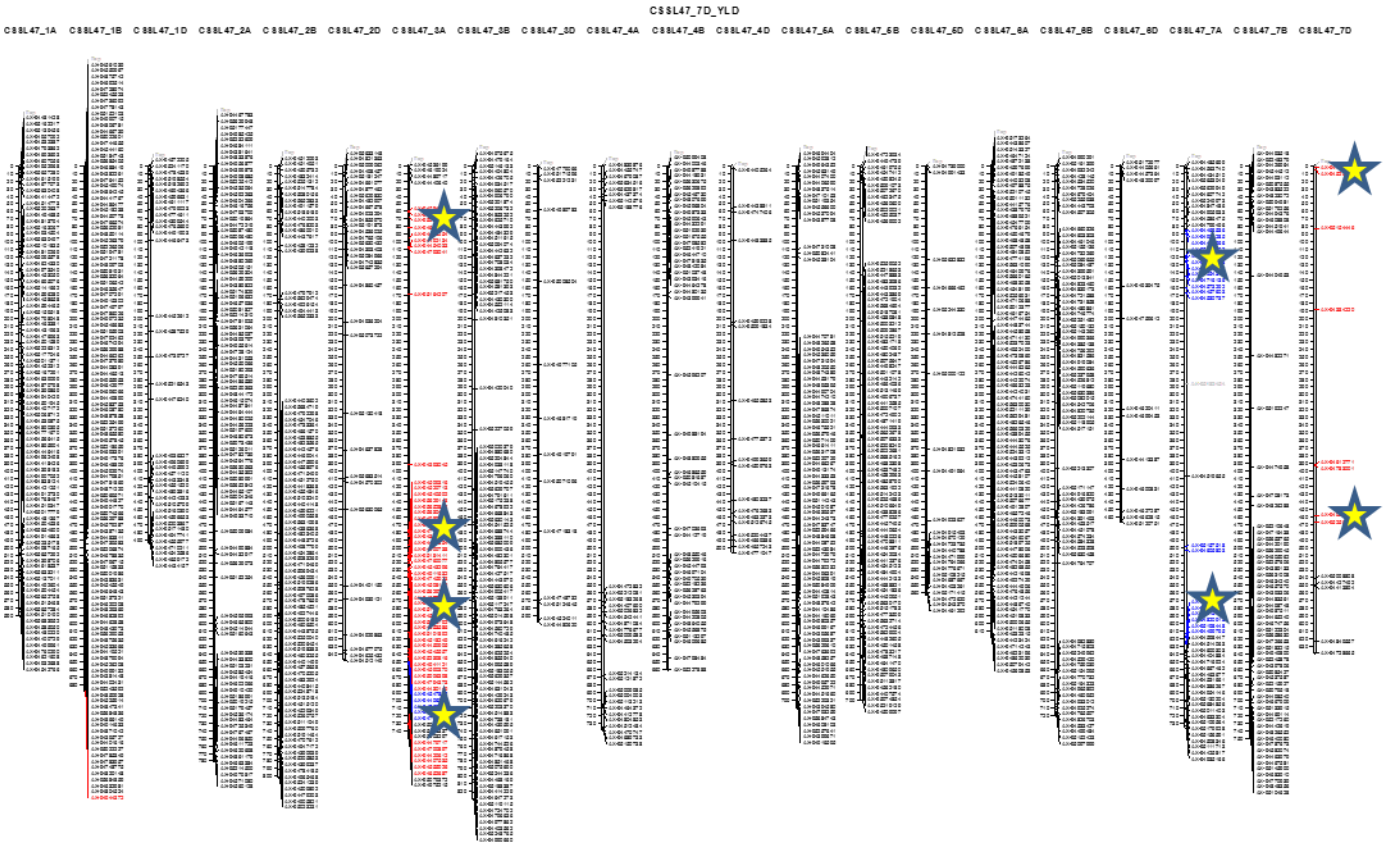


Figure 3 Whole genome map of the 21 chromosomes from CSSL #47 which carries a YLD QTL on 7D. Map was generated using the IWGSC RefSeq v1.0 and shows the Mb position of the Axiom markers colour-coded (Avalon, Cadenza, Heterozygous). The yellow stars denote introgressed regions identified with KASP markers.

Analysis of all the maps indicated that the maximum possible coverage, with the material available, could be obtained from 57 CSSL lines; this will give almost complete coverage of Avalon segments in the Cadenza background but unfortunately a lower coverage of Cadenza segments in the Avalon background. Seed from each of the 57 CSSL BCF2 populations were sown and leaf material collected from 94 plants for DNA preps and genotyping; almost 6000 plants in total (see **Figure 4**)!

Meanwhile the DNAs have been genotyped for all markers to their introgressed regions. The data for chromosomes 1 – 5 has been analysed with that for chromosomes 6 and 7 due shortly. Genotyping shows the extent of introgressed regions and variety of recombination events across each chromosome segment which can subdivide these regions (i.e. Chr 3A in CSSL47, see **Figure 5**).

Figure 4. Trays of CSSL seedlings growing in the glasshouse, prior to leaf harvesting.

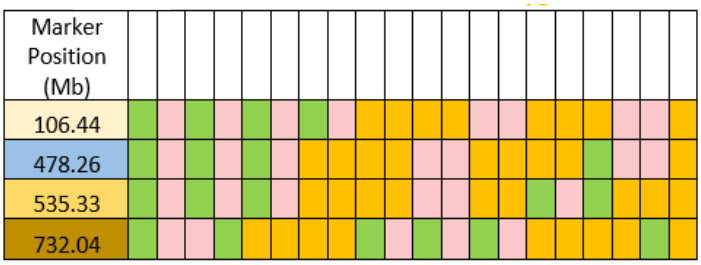


Figure 5 Each column represents a CSSL line (or lines). The blocks indicate the genotype of these lines for each of four markers on Chr3A at the Mb position shown (see also Figure Y). Avalon = green, Cadenza = pink, heterozygous = yellow

When the full genotyping has been compiled it will be possible to browse the data on the WGIN website to select, where possible, lines which have specific chromosomal segments. In the meantime, Axiom maps of the 94 original CSSL lines will be on the WGIN website from April. BC_3F_3 seed were collected from a single ear from each of the genotyped plants. Glasshouse bulking of the most useful lines will be carried out early 2019 but very small aliquots of seed may be available before then. The seed will eventually be deposited in the Germplasm Resources Unit (GRU) at the JIC.

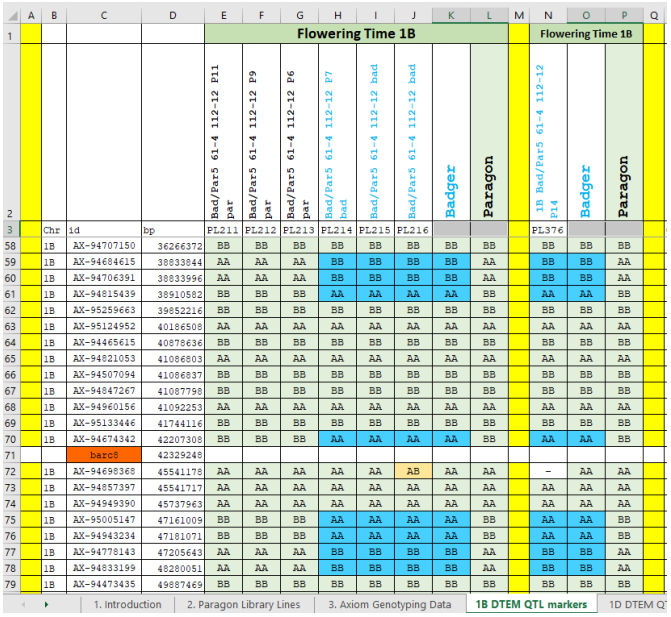
- **The Paragon Library data is now available**

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. NILs are currently available for multiple alleles of: Rht-D1, Rht-B1, Rht8, Lr19, 1BL.1RS, Yield (7B), Grain Size (5A, 7A) and more than 10 Heading Date QTL. Further lines will be added in the future.

The Paragon Library lines have been genotyped on the Axiom® 35K Breeders' Array and most of this data can now be downloaded as an Excel file from the WGIN website (http://www.wgin.org.uk/resources/Paragon_library.php)

The genotyping data was processed and only markers polymorphic between Paragon and the introgressed segments have been included. The lines that have been genotyped are grouped by QTL or other genetic feature, and the positions of microsatellite and KASP markers used to identify the QTLs are indicated. The genotyping data is laid out so the chromosome number, Axiom marker name and its position in the IWGSC RefSeq v1.0 can be easily viewed on a single screen beside each group of lines (see **Figure 6**).

Seed collected from the genotyped plants are now available for distribution on request and we hope they will become available through the **Germplasm Resources Unit** (GRU) at JIC.



	Chr	id	bp	Bad/Par5 61-4 112-12 P11 par	Bad/Par5 61-4 112-12 P9 par	Bad/Par5 61-4 112-12 P6 par	Bad/Par5 61-4 112-12 P7 bad	Bad/Par5 61-4 112-12 bad	Bad/Par5 61-4 112-12 bad	Badger	Paragon	1B Bad/Par5 61-4 112-12 P14	Badger	Paragon
				Flowering Time 1B								Flowering Time 1B		
3	Chr	id	bp	PL211	PL212	PL213	PL214	PL215	PL216			PL376		
58	1B	AX-94707150	36266372	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
59	1B	AX-94684615	38833844	AA	AA	AA	BB	BB	BB	BB	AA	BB	BB	AA
60	1B	AX-94706391	38833996	AA	AA	AA	BB	BB	BB	BB	AA	BB	BB	AA
61	1B	AX-94815439	38910582	BB	BB	BB	AA	AA	AA	AA	BB	AA	AA	BB
62	1B	AX-95259663	39852216	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
63	1B	AX-95124952	40186508	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
64	1B	AX-94465615	40878636	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
65	1B	AX-94821053	41086803	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
66	1B	AX-94507094	41086837	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
67	1B	AX-94847267	41087798	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
68	1B	AX-94960156	41092253	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
69	1B	AX-95133446	41744116	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
70	1B	AX-94674342	42207308	BB	BB	BB	AA	AA	AA	AA	BB	AA	AA	BB
71		barc8	42329248											
72	1B	AX-94698368	45541178	AA	AA	AA	AA	AA	AB	AA	AA	-	AA	AA
73	1B	AX-94857397	45541717	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
74	1B	AX-94949390	45737963	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
75	1B	AX-95005147	47161009	BB	BB	BB	AA	AA	AA	AA	BB	AA	AA	BB
76	1B	AX-94943234	47181071	BB	BB	BB	AA	AA	AA	AA	BB	AA	AA	BB
77	1B	AX-94778143	47205643	AA	AA	AA	BB	BB	BB	BB	AA	BB	BB	AA
78	1B	AX-94833199	48280051	AA	AA	AA	BB	BB	BB	BB	AA	BB	BB	AA
79	1B	AX-94473435	49887469	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB

1. Introduction | 2. Paragon Library Lines | 3. Axiom Genotyping Data | 1B DTEM QTL markers | 1D DTEM Q

Figure 6 Screenshot of a page from the **Paragon Library** Genotyping Data Excel file showing the 1B flowering time QTL region from Badger, compared to Paragon. Marker barc8 was used to map this QTL.

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

Nitrogen Use Efficiency (NUE) and Quality Resilience (RRes)

- **New WGIN Objectives for the 2019 Diversity Trial**

The WGIN Diversity field experiment has run continuously since 2004. Each year a panel of wheat varieties have been grown at different levels of nitrogen. For most of the period the panel has been 25-30 varieties grown at zero, 100, 200 and 350kg N/ha, as well as a full crop protection regime. The panel has changed slowly over the course of the project, most years taking on 1-2 new varieties and dropping some less popular, but maintaining a core of about 12 varieties throughout, including some varieties from the 1960-1990 period.

As well as providing data on yield and nitrogen use, the experiment has been used for other research; since 2014 it has provided an ideal resource for developing drone phenotyping technology, as reported in previous newsletters.

For the new WGIN project, it was decided to make further changes to the design – the current experiment had produced good data over many years and it was felt change was due. The nitrogen rates of 100, 200 and 350 have been kept, but the zero rate dropped – in practice it is unrealistic: it provides useful data for nitrogen use efficiency studies, but is not a rate encountered commercially. Also, the suite of varieties has been reduced to 20.

The biggest change has been the introduction of a fungicide treatment - each variety will be grown at standard and reduced fungicide/ no insecticide input regimes, at each of the three nitrogen levels. The reduced regime will be managed to allow diseases and pest infestations to occur, but to try to not let them reach severe levels.

Figure 7 Aerial view of the WGIN Diversity Trial 2018 on the Rothamsted farm

The new experimental design will provide data on disease and pest resistance and tolerance across the varieties, interacting with nitrogen inputs. In addition, there is a key part of the project looking to develop sensing technologies, including drone-based solutions to detect and quantify disease levels. The drone approach faces several challenges – the ability to acquire high resolution images from a moving drone, taken at precise points. A major challenge, and part of the project is to see whether disease presence within a leaf canopy can be detected – imaging disease at a canopy surface should be possible, but in practice it is more important to be able to identify disease occurrence below the surface of the canopy.

Beyond this, and the scope of WGIN, will be automated identification and quantification of disease within images, however it is important to develop the image capture capability first – without this the identification will not be possible.

It will be possible to view the new WGIN Diversity Trial site and learn about the use of drone technology on the **D**esigning **F**uture **W**heat **Open Day** (June 20th 2019, see 'Events' section for details).

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

Resilience to Aphids (RRes)

Barley Yellow Dwarf Virus (BYDV) has been on our mind due to the changes in pesticide regulations and much speculation goes on regarding the effect the neonicotinoid ban will have on BYDV incidence and yield effects in coming years. See WGIN press release November 2018 https://www.rothamsted.ac.uk/news/future-wheat harvests -very-vulnerable-disease-warn-experts.

To start answering this we are monitoring aphids and looking at BYDV infection rates and the effect of the virus on the diversity trials at Rothamsted. **We also intend to work with farmers and/or breeders who are happy for us to do some sampling in their fields**. This would not affect their wheat production in any way as we only need small samples from a few plants per field. For info, please email Gia Aradottir at gia.aradottir@rothamsted.ac.uk or message us on Twitter (**@WheatGIN**).

Figure 8 Searching for autumn populations of cereal aphids in the diversity trial at Rothamsted in November 2018

Laboratory experiments are underway to assess the susceptibility of wheat to BYDV, to inform farmers about breeders' choices when looking for resilience to the disease. We are starting with the varieties that form part of the diversity trials and will be sharing this data as soon as this becomes available.

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

Foliar Disease (RRes)

- **Disease Resilience**

New activities within WGIN4 are aimed at improving the resilience of the UK wheat crop against fungal pathogen attack by exploiting novel genetic resources and traits. In previous WGIN newsletters we have reported on our studies exploring septoria, yellow rust and take-all root disease resistance from within the Watkins landrace collection and the ancestral wheat relative *Triticum monococcum*. New experiments within WGIN4 include the investigation of a novel prolific rooting phenotype (3N) and exploring the potential of wheat *mlo* mediated powdery mildew resistance under field conditions.

- **3N ancestral introgression rooting trait**

Below ground rooting traits have previously been under explored by the wheat breeding community. However novel rooting phenotypes could help plants to more efficiently utilise water and nutrients in a range of environments and could also be used to improve performance/tolerance against root diseases and thereby improve overall root health. At the John Innes Centre, 3N ancestral introgressions from *Aegilops uniaristata* into the hexaploid bread wheat Chinese Spring show a prolific rooting phenotype and aluminium tolerance (Clare Lister and Simon Griffiths, unpublished). Within WGIN4 the effect of the prolific rooting 3N ancestral introgression trait on take-all root disease will be explored. Initially the ancestral introgressions will be phenotyped and compared to wild type Chinese Spring in a five-week seedling bioassay with artificial take-all fungal inoculum addition. Plants will be examined for both root disease severity, plant growth characteristics and root architecture to explore the effect on both disease severity and disease tolerance. After further seed multiplication the lines will also be phenotyped under high root disease pressure in 3rd wheat field trials on the Rothamsted Farm to explore their performance under field conditions.

- **Wheat *mlo* mediated resistance against powdery mildew**

Loss-of-function mutations in the *Mildew resistance locus o* (*mlo*) gene have provided recessively inherited resistance against powdery mildew (*Blumeria graminis* f. sp. *hordei*) in barley (*Hordeum vulgare*) (reviewed by Kusch and Panstruga, 2017). *mlo*-based resistance is race nonspecific (i.e. effective against all races) and has been widely used in commercial spring barley breeding programmes to provide durable resistance against powdery mildew across multiple decades. *mlo* mediated resistance has now been described in many other plant species including both monocots and dicots, for example tomato, tobacco, apple, cucumber, melon and pea (Kusch and Panstruga, 2017). Both positive and negative pleiotropic effects have been associated with barley *mlo* mutants. Le Fevre et al. (2016) reported that young leaf sections from barley *mlo* plants were more resistant to the oomycete pathogen *Phtyophthora palmivora*. However, this was not maintained in older leaf sections or roots. The barley *mlo* mutations have also been associated with early leaf senescence and increased susceptibility to nonbiotrophic pathogens such as *Magnaporthe grisea* and *Fusarium graminearum*.

On the current UK AHDB recommended list for winter wheats (2018/19), seven varieties have only a low to modest disease resistance rating of between 3 to 5 for mildew. With the EU about to ban the widely used mildew preventing fungicide, chlorothalonil, there is concern over the vulnerability of the wheat crop to powdery mildew.

Using the **WGIN 1 Cadenza TILLING population** (http://www.wheat-tilling.com), Ralph Panstruga's group in Aachen, Germany, have generated the equivalent mutants in hexaploid bread wheat by screening the TILLING population to identify mutations in the three homoeologous genes and combine these through conventional breeding. In seedling glasshouse tests, high levels of resistance to powdery mildew were identified in the triple mutants and some double mutant lines (**Figure 9**) (Acevedo-Garcia et al. 2017).

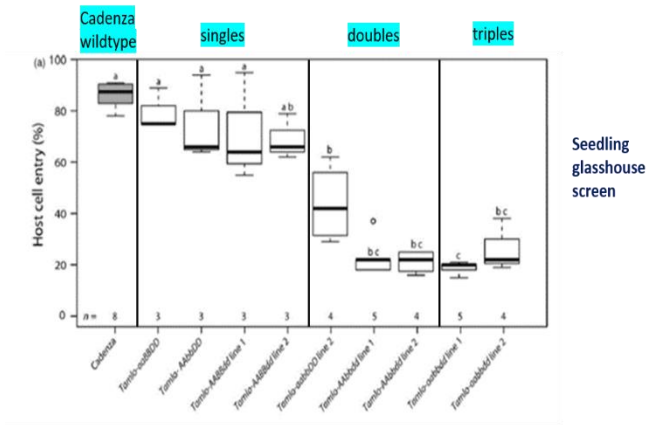


Figure 9 Powdery mildew host cell entry scored at 72 hours post infection for single, double and triple *Tamlo* mutants compared to wild type Cadenza. Figure reproduced from Acevedo-Garcia et al. (2017).

Powdery mildew resistance is currently being tested under field conditions in Germany by the Panstruga group. Within WGIN4 we will be phenotyping the best performing double and triple mutants under field conditions for susceptibility/ resistance to other fungal pathogens. This will include the rusts, septoria and fusarium. In addition, overall plant growth and development (including leaf senescence) will be explored to detect possible pleotropic effects. By this route we will be able to evaluate thoroughly the value of this new powdery mildew resistance to wheat breeders.

In the first field season (2018) we carried out field multiplication of the 14 triple and 4 double *mlo* mutants in small single replicate plots with 30 seeds of each mutant. Foliar disease observations and plant development phenotypes e.g. ear emergence and plant heights, were recorded. Given the extreme drought conditions during summer 2018 the overall levels of foliar disease were very low and leaves prematurely senesced across the field trial. However, we observed greater leaf senescence for wild type Cadenza than the double and triple mutants (**Figure 10**).

Grain was harvested from each of the plots during August 2018 and fully replicated field trials were sown in October 2018 for the 2018/19 field season. Slow plot emergence was noted for some mutants during autumn 2018. However, this emergence trait does not seem to be related either to the independent original mutant line, or the specific single nucleotide substitutions present at the *mlo* locus. In spring and summer 2019, regular foliar disease observations will be made, and growth and development closely monitored to fully evaluate the double and triple mutant field performance compared to wild-type Cadenza.

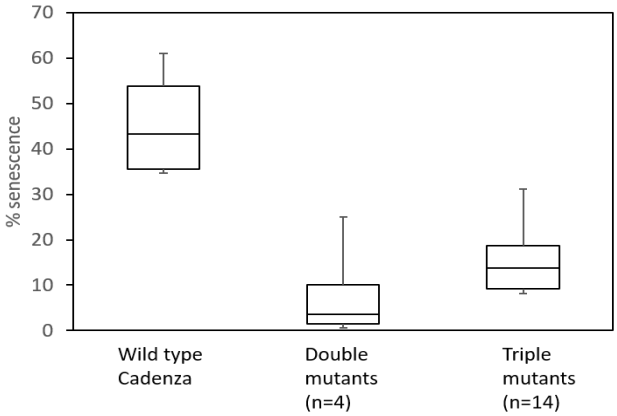


Figure 10: Percentage leaf area naturally senesced (not associated with any disease symptoms) for leaf 2 assessed during milk development in July 2018. Single replicate of each *mlo* mutant, 10 tillers assessed per plot. N.B. Leaf 3 was fully senesced for all the mutants and wild type Cadenza.

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For further information on this aspect of the WGIN project contact Vanessa McMillan (vanessa.mcmillan@rothamsted.ac.uk) or Kim Hammond-Kosack (kim.hammond-kosack@rothamsted.ac.uk) at RRes.

Introgression of *Triticum monococcum* Into Hexaploid Wheat (RRes)

As reported in the 2018 Newsletter (available online) the current approach to introgression of *Triticum monococcum* into hexaploid wheat employs two tetraploid wheat cultivars, Kronos and Hoh501, as bridging species. **Table 4** shows the different stages of this crossing strategy as a reminder.

Table 4: The different stages of *Triticum monococcum* introgression into hexaploid wheat. Tdur= *Triticum durum,* Tm= *Triticum monococcum*, Taes=*Triticum aestivum*, F1C=F1 complex, BC=back cross

Stage	Crosses (♀ x ♂)	ploidy
Stage 1	Tdur x Tm = F1_hybrids	triploid
Stage 2	F1_hybrids x Taes = F1C	pentaploid
Stage 3	F1C x Taes = F1C_BC1	hexaploid(ish)
Stage 4	F1C_BC1 x Taes = F1C_BC2	hexaploid(ish)
Stage 5	F1C_BC2 x Taes = F1C_BC3	hexaploid

At the time of preparing this newsletter, stage 3 has been completed and 39 grains were successfully germinated and the plants are just finishing the 8 week vernalisation period (details below). **Figure 11** shows the five F_1C plants used for pollination with Paragon, after emasculation of spikelets. Some spikelets were not emasculated and left to self-fertilise (F_1C_S).

Figure 11 The 5 F1-Complex (F1C) plants generated previously and used for backcrossing with Paragon (stage 3) to generate F1C_BC1 grains.

Two of the three F_1C plants generated with Hoh501 as the female parent are considerably taller than the tetraploid parents (not shown), and this would be expected in line with the 'tall height' phenotype of *T. monococcum* plants. However, it is interesting to note the difference in height between the two F_1C#86 plants. These plants were generated by crosses two florets on the same wheat ear. But this difference is not unexpected because at this stage there is still segregation occurring and two florets could give two different phenotypes (David Feuerhelm, Syngenta, pers. comm.).

The results for stage 3 are shown in **Figure12**. Considerably more grains were generated with Hoh501 than with Kronos as the original female parent in stage 1, and also the fertility of these plants is higher with F_1C#86-1 and F_1C#94, in particular, showing just over 20% fertility. Considering that the fertility in the previous stage was around 0.5%, this equates to a 40 fold increase from stage 2 to stage 3.

In the next stage (stage 4), it is expected that fertility should increase further and reach the same level as crossing between two hexaploid parents by stage 5.

Currently, 28 $F_1C\_BC_1$ plants and 11 F_1C_S plants are coming out of vernalisation. We would expect a large enough number of $F_1C\_BC_2$ grains to be generated in stage 4 to allow testing for introgressed resistance to Take-All, Yellow Rust and Septoria fungal diseases as well as aphid pests.

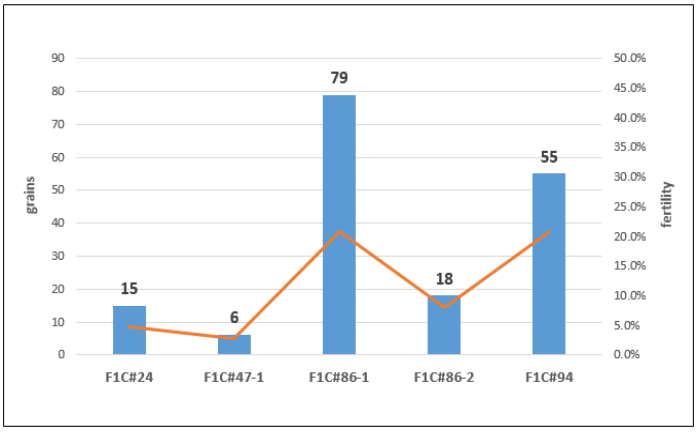

Figure 12 Generation of $F_1C\_BC_1$ grains (stage 3). Blue = number of grains generated with each F1C plant, orange = fertility [grains obtained/stigmas pollinated] (%)

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

Section 3 Previous Events

- The Rothamsted Research **Festival of Ideas** Open Science weekend took place from **22nd to 24th June 2018** to celebrate the **175th anniversary of Rothamsted**. Between 8,500-9,000 visitors were hosted by Rothamsted (https://www.rothamsted.ac.uk/175). The '**Wheat Genome in Action**' interactive game based on the **WGIN promotome capture experiment** was devised by Michael Hammond-Kosack. The 21 wheat chromosomes (projected onto a 3mx5m Velcro covered screen as the A, B or D genomes) were reproduced to the scale of 1m=330Mbp, upon which the centromeres and 70 genes from the 10 trait categories were placed as coloured bands. Each player threw Velcro covered balls with the aim of hitting any 'trait gene' on each of the 3 sub-genomes. The function of each 'hit gene' was then explained in both scientific and layperson terms. Each participant that hit a gene then received a small prize by randomly sampling a mixed box of WGIN diversity trial wheat grain, and with a few instructions was encouraged to grow wheat in their garden or allotment. Participants that hit the same gene on all three genomes received a dried wheat tiller as a prize as well. In total, approximately 450 people (mostly, but not exclusively, children) successfully played the game / received a prize and about the same number of visitors watched on. **Figure 13** shows the layout and some participants as well as assistants of this game.

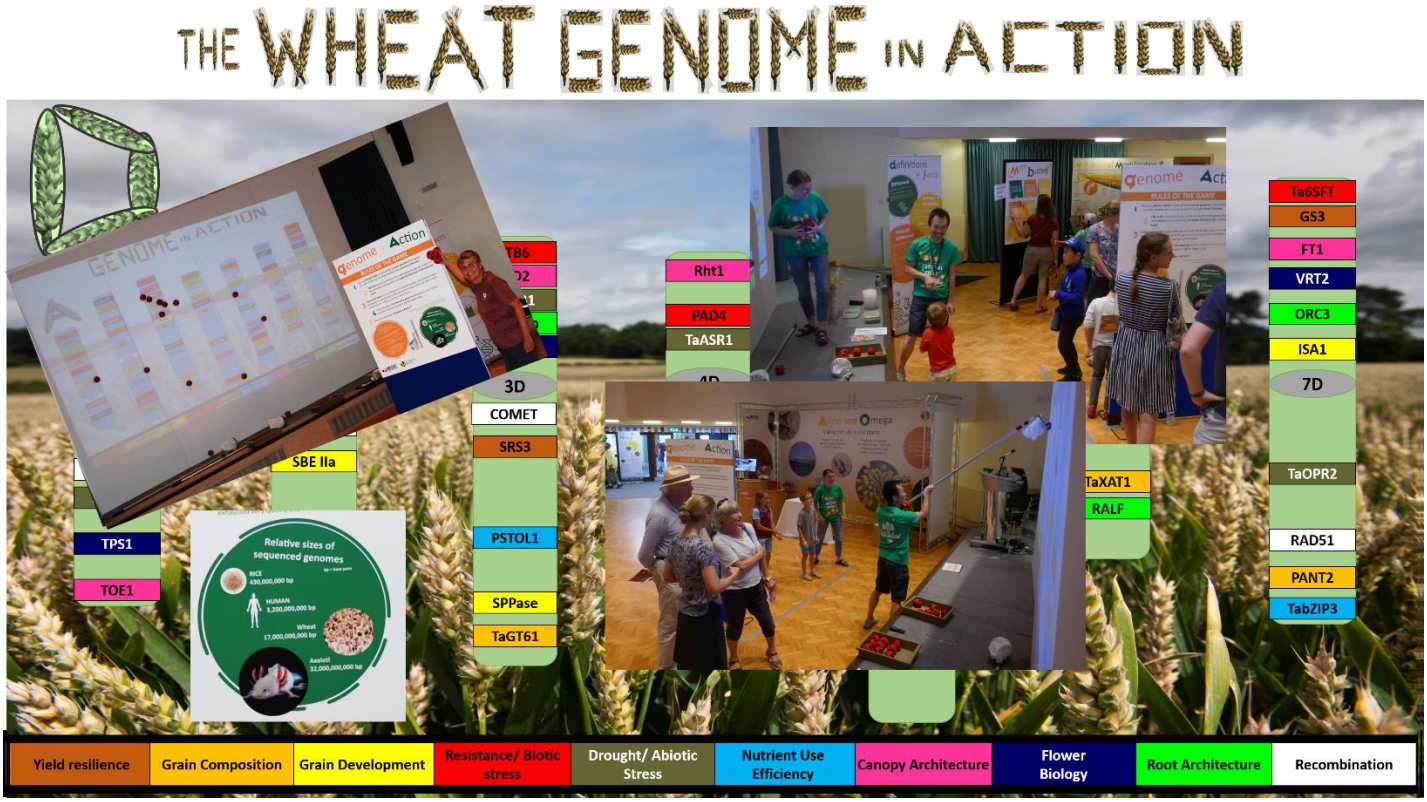

Figure 13 The 'Wheat Genome In Action' game showing here the chromosomes of the D genome (green bars) and A genome (inset picture with projection and Velcro balls) with the centromeres (grey ellipses) and some of the colour-coded trait genes, covering all 10 WGIN Promotome trait categories.

- A second display, again inspired by WGIN, explained the **origin of modern wheat** and the species that are present in the primary, secondary and tertiary wheat improvement pools. This display consisted of the beautiful poster designed by Dr. Kostya Kanyuka (RRes) (**Figure 14**) but also live plants of most of the Genepool cultivars. Visitors were also shown either a video or a live demonstration of how wheat crossing is done.

- The **16th WGIN Stakeholders' Meeting** was held jointly with the BBSRC Designing Future Wheat project @ Rothamsted Research on **November 16th 2018**. The panel discussion focussed on '**Loss of chemistry – Implications for the Wheat Crop**'. Some of the presentations as well as the agenda can be viewed on the WGIN website (http://www.wgin.org.uk/stakeholders/stakeholdermeetings.php).

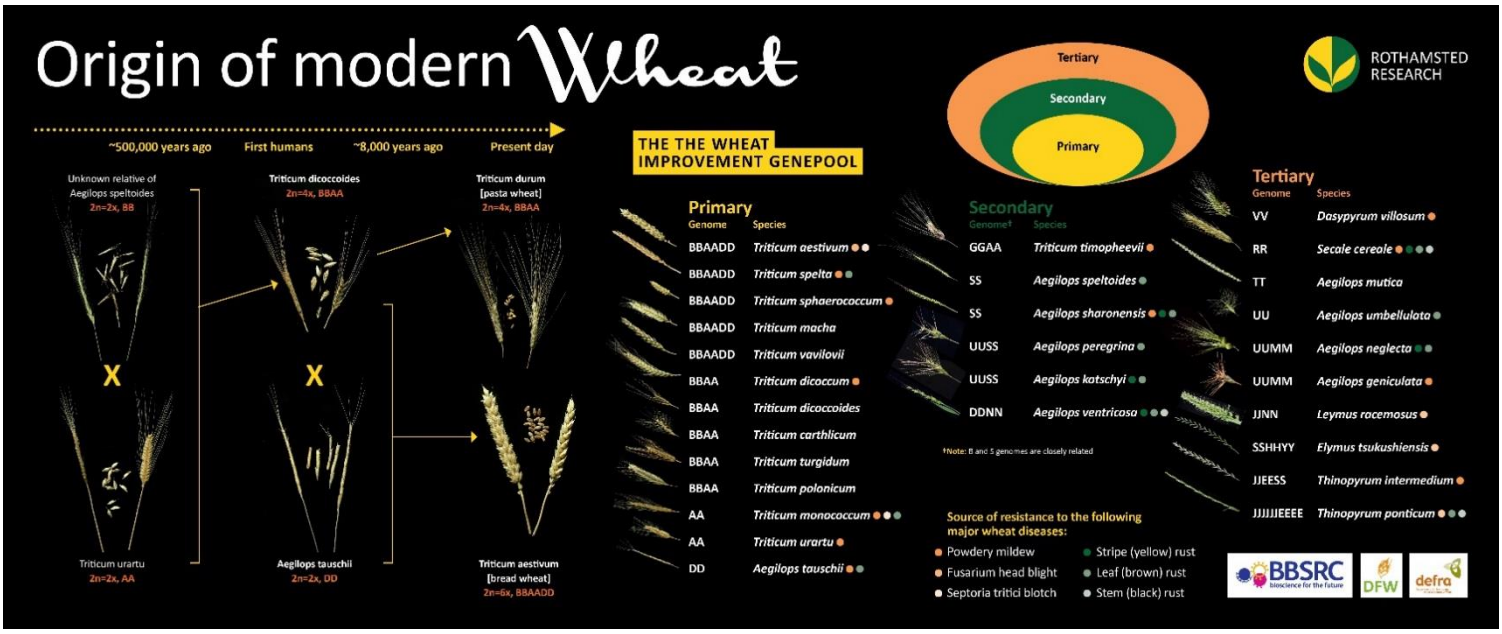

Figure 14 The 'Origin of modern wheat' poster designed by Kostya Kanyuka for the Festival of Ideas, on permanent display at Rothamsted.

Section 4 News

- A selection of WGIN experiments, trait discoveries and new technologies will be on display at the annual **Cereals event** in Lincolnshire (12th -13th June) at the Rothamsted Research Exhibit.

- During the **Designing Future Wheat Open Day on Thursday 20th June 2019 at Rothamsted Research** we will display the new WGIN variety diversity trial featuring NUE, crop protection and UAV monitors to detect / monitor pests and pathogens. There will also be displays on take-all root disease, Septoria leaf blotch disease, aphids and BYDV and the new sources of resistance identified, a glasshouse display of the Cadenza Tilling population for novel trait discovery and a display on the DFW breeders tool kit into which some of the new WGIN traits are entering for field evaluation by the commercial breeders. You can still register for the DFW Open Day here: https://www.eventbrite.co.uk/e/dfw-open-day-tickets-56914432600

- The WGIN twitter handle is **@WheatGIN**. Since Feb 2018 this has been used to inform our followers on grant successes, WGIN publications and press releases, and information about the forthcoming Stakeholder events. Since Jan 2019, each week we have featured a different WGIN article published sometime in the past 5 years.

- The WGIN annual Stakeholders' Meeting will take place sometime in November 2019 at Rothamsted Research. We have already decided that this year's panel discussion will be on '**Loss of Insecticides**' with a focus on insect pest control as well as the virus species transmitted by insects. If you are interested in becoming a panellist please contact Peter Shewry (peter.shewry@rothamsted.ac.uk) or Mike Hammond-Kosack (wgin.defra@rothamsted.ac.uk).

Section 5 Publications 2017 to 2019

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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, Andrew Riche, Vanessa McMillan, Gia Aradottir, Michael Hammond-Kosack (**RRes**); Clare Lister and Simon Griffiths (**JIC**)