

WGIN4 Updates 2020

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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 million hectares) and is more valuable than any other arable crop in the UK. The **Wheat Genetic Improvement Network (WGIN)** started in 2003 and 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 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 consists of four work packages (WPs) (**Figure 1**). WP1 focusses on further enhancing the networking and communication activities. Through the newly formed project management group (PMG) and the research advisory group (RAG), we have successfully aligned 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>). As a result a new joint GINs website is available so that stakeholders can track the planned and ongoing activities, publications and other outputs across a wide spectrum of crops. This joint GINs website established in 2020 is available at <https://defracropgenetics.org>.

Within WGIN4 the three interconnected research work packages (WP2, WP3 & 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 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 four 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, and the **National Institute of Agricultural Botany (NIAB)** for screening new sources of resistance with specific races of yellow rust.

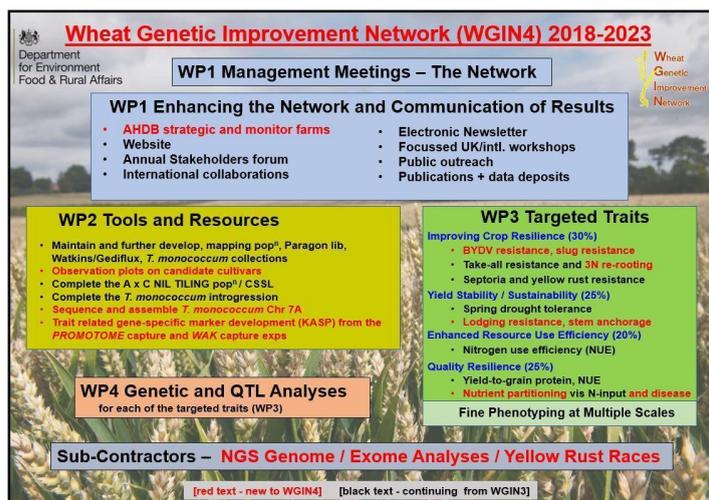


Figure 1 The organisation of WGIN4. All red text highlights are new additions compared to WGIN3.

Section 2 Research Updates

WGIN4 at the JIC

● Yield Stability and Sustainability Traits; Drought Tolerance, Lodging and Anchorage

Wheat growing conditions are becoming even more difficult due to variable climate conditions, in particular volatile temperatures and unpredictable rainfall. We only have to look at the last two growing seasons and autumn 2019 to spring 2021 to see how challenging this is! We are working to produce wheat

which is able to deliver a consistently high yield in these variable weather conditions, and in the different UK wheat growing areas. Improved drought tolerance is one route to increased yield stability and resilience to reduced rainfall. Spring drought is now a common feature of the UK climate, April 2020 and 2021 being classic examples of this, and these adverse conditions can severely affect grain number and therefore yield. In addition, extreme weather conditions, such as heavy rain and strong winds, will inevitably lead to increased lodging and subsequent crop loss.

● Lodging and Anchorage

At the JIC we are continuing to **concentrate on yield stability traits**, including the effect of spring drought, but also examining lodging and stem (essentially root) anchorage. In 2018 we assembled a 'Drought, Anchorage and Lodging Panel' (**DALP**) of lines which may potentially have drought tolerance/sensitivity and/or lodging resistance/sensitivity and may also show variation in anchorage strength. These lines come from many sources including derived materials generated in WGIN, the Designing Future Wheat (DFW) Breeders' Toolkits (2017 and 2018), UK varieties from breeders, CIMMYT varieties from the Semi-Arid Wheat Yield Trial (SAWYN panel) and a set of the Stress Adapted Trait Yield Nurseries (SATYN) panel, the Rothamsted WGIN Diversity Panel, Watkins, promising Recombinant Inbred Lines (RILs) from the Paragon x Garcia Drought Trial (PxG DT), the parents of existing mapping populations and of crosses between Recommended List (RL) lines (see the 2019 WGIN newsletter for details).

We drilled the DALP collection as 1 m² plots in autumn 2018 for seed bulking and initial phenotypic observations, including lodging assessments, as well as heading date and plant height. We also used these plots to develop a method to measure root anchorage. Root anchorage has rarely been assessed previously. Preliminary anchorage tests indicated a wide range of anchorage strength within a variety which would therefore require a high number of tests per plot. It was clear that a simple, quick method for the *large-scale* screening of single plants was required but none were available – **unless any readers know otherwise!** With a nod to Heath Robinson we developed a method using a pair of car jump lead

clips, some agricultural twine and a range of pull force gauges (from 20N to 200N)(Figure 2).



Figure 2 Anchorage strength assessment method

In very late autumn 2019, and under the difficult conditions familiar to farmers and wheat researchers alike, we drilled two 6 m replicates of the DALP panel as spaced plants (approximately 5 cm apart). A single replicate was to be additionally irrigated in the spring, if spring drought occurred, and the other replicate to be rain-fed only. The degree of lodging was to be recorded in these lines as well as an assessment of anchorage after anthesis, using our developed method. So... while we were all in Covid-19 lockdown there was the most gloriously (for a drought researcher!) dry April this year but of course we were unable to perform the differential irrigation. If possible we will try to investigate the effects of the differential irrigation on these lines during grain fill but at the very least we will ensure the seeds are harvested for re-drilling in autumn 2020. In addition, we will have the opportunity to modify our trial design. **Update spring 2021:** We have re-drilled this trial but as conventional 6m yield plots. The spaced plants lead to excessive tillering in many lines and therefore very dissimilar to a normal field of wheat! We were able to score lodging in most lines in 2020 but not anchorage. We have been irrigating one rep of DALP since April and hope to do a full analysis of this trial.

As mentioned below we felt an additional year of the Paragon x Garcia Drought Trial was essential, which has delayed our exploration of new populations. To make up for this we actually explored more populations than originally planned, we drilled a single rep of 1m² plots, consisting of 466 selected individuals from 50 Nested Association Mapping (NAM) populations of crosses between varieties or Watkins lines (some of which appear in DALP) with Paragon (Table 1). We aim to collect heading and height data as usual, and make an assessment of lodging for both these trials, if possible. The second

trial should identify candidate populations for drilling in autumn 2020. **Update spring 2021:** We were able to score lodging in these lines in 2020 as well as developing a simple field-scoring method for assessing stem strength (Figure 3). We selected **Par x Watkins 110** and **Par x Joss Cambier** for replicated lodging trials, as 1 m² and 6 m² plots, respectively.

Table 1 List of the Paragon x Watkins and Paragon x Variety populations from which individuals were selected. (Par = Paragon)

| | | |
|-------------------|-------------------|---------------------|
| Par x Watkins 7 | Par x Watkins 308 | Par x Bacard Kachu |
| Par x Watkins 32 | Par x Watkins 324 | Par x Baj |
| Par x Watkins 42 | Par x Watkins 387 | Par x Charger |
| Par x Watkins 94 | Par x Watkins 451 | Chris x Par |
| Par x Watkins 103 | Par x Watkins 496 | Par x Courtot |
| Par x Watkins 110 | Par x Watkins 507 | Par x Fiorello |
| Par x Watkins 126 | Par x Watkins 560 | Par x Glasgow |
| Par x Watkins 139 | Par x Watkins 670 | Par x Joss Cambier |
| Par x Watkins 145 | Par x Watkins 694 | Par x MISR1 |
| Par x Watkins 199 | Par x Watkins 700 | Par x Pamyati Aziva |
| Par x Watkins 218 | Par x Watkins 705 | Par x Pfau |
| Par x Watkins 219 | Par x Watkins 731 | Par x Starke |
| Par x Watkins 224 | Par x Watkins 740 | Par x Super152 |
| Par x Watkins 231 | Par x Watkins 747 | Par x Waxwing |
| Par x Watkins 239 | Par x Watkins 749 | Par x Wyalkatchem |
| Par x Watkins 246 | Par x Watkins 777 | |
| Par x Watkins 291 | Par x Watkins 912 | |
| Par x Watkins 305 | | |

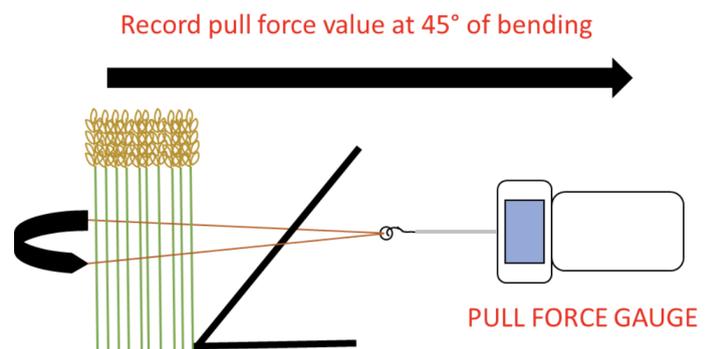


Figure 3 The force required to bend 10 stems to an angle of 45°.

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

UK wheat is sensitive to spring drought as it coincides with the beginning of stem extension (GS31) when grain number starts to be determined and is likely to cause yield reduction though reduced grain

number. As described in previous WGIN newsletters, we have been searching 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 trials, at the JIC farm, Church Farm, have consisted of two randomised replicates each of the 177 Paragon x Garcia (PxG) RILs, plus nine lines from the Paragon Library (see below), with Paragon and Garcia controls, arranged in 'Not Irrigated' (NI) and 'Irrigated' (IR) 6 m² plots. The scheduled trials ran from October 2015 – August 2018 but due to the lack of spring drought in both 2016 and 2018 it was considered essential to run the trial for a 4th year and hope for spring drought in 2019! This strategy proved successful as the drought in 2019 was slightly worse than 2017, in terms of the amount of rainfall, but the effects of the 2017 drought were more dramatic due to the lightness of the soil of that field (Riverside). Our trials are rotated on a 3-year cycle so we are dealing with differences in both the amount of rainfall and the soil type, shown in **Table 2**.

Table 2 Weather and soil conditions for the four Paragon x Garcia drought trials

| Trial years | Spring | Summer | Field | Soil Type |
|-------------|----------------|-----------------------|----------------|------------|
| 2015-16 | Spring rain | Little summer drought | Opposite Drier | Sandy Loam |
| 2016-17 | Spring drought | Some summer drought | Riverside | Very sandy |
| 2017-18 | Spring rain | Severe summer drought | Football | Very sandy |
| 2018-19 | Spring drought | Some summer drought | Opposite Drier | Sandy Loam |

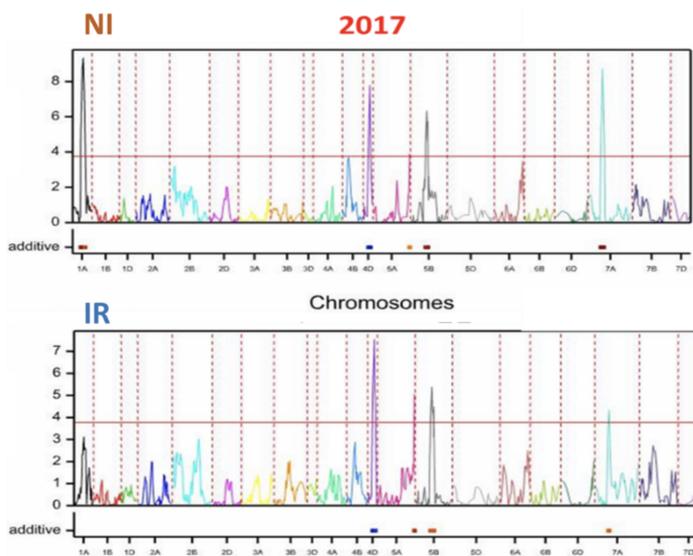


Figure 4 Graphs of the QTL mapping output for TGWT in 2017. Upper panel from the Not Irrigated (NI) plots and the lower panel from the Irrigated plots (IR). The peaks show where there is a significant difference in TGWT between RILs which are Garcia or Paragon. To be significant the peak must be above the orange horizontal line, just below 4 on the scale bar. The individual chromosomes (1A-7D) are shown at the bottom of the graphs, above which are orange or blue bars indicate whether Garcia (orange) or Paragon (blue) has the highest value for TGWT.

Data for yield traits (harvest yield YLD, Thousand Grain Weight TGWT, Specific Weight SW and grains/m²) were collected, as well as heading date and plant height. The yield data was used for QTL mapping; a calculation using the quantitative yield data and DNA marker data from the Paragon x Garcia RILs to identify specific chromosome regions from Garcia carrying drought tolerant genes. **Figure 4** shows the QTL results of TGWT for 2017 and very similar results were obtained in 2019. The decision to run the 4th year of the PxG drought trials has been vindicated by the results obtained.

For TGWT the most significant QTL is on Chr 1A as it only shows in Garcia under NI conditions. QTLs on Chr 5A, 5B and 7A are present in both NI and IR plots; 5B and 7A are reduced in the IR plots and are therefore also of interest, while the QTL on 5A is increased in IR. The QTL on Chr4D is *Rht1* from Paragon. The QTL on 1A is one of the two loci already included in a NIL production programme. **Update Spring 2021:** We have completed a full analysis of the data from the four years of drought trials. We now think that **the most import QTL is for yield and found on 2B in Garcia**. The data suggest that this Garcia allele can increase both grain size *and* grain helping plants survive both spring and summer drought.

- **Delivery of other beneficial loci into UK wheat breeding**

We have a number of other on-going projects to generate resources which should deliver beneficial loci into UK wheat breeding, including two studies which examine the effect of combining semi-dwarf genes together, and with vernalisation genes in Paragon.

- ***Rht1* x *Rht8***

Crosses have been made between semi-dwarf Paragon Library lines (see below); *RhtB1*, *RhtD1* and *Rht8*. All three genes cause a reduction in stem elongation which increases resistance to lodging, with *RhtB1* and *RhtD1* also showing an increase in yield. *Rht8* may help the growth of plants in nutritionally poor or high temperature conditions.; *RhtB1* or *RhtD1* was crossed to *Rht8*, as well as *RhtB1* x *RhtD1* and these lines are in a year-3 yield trial. The results of the previous two years trials show a higher yield in lines which carry both *Rht1* and *Rht8*, compared to *Rht8*,

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

- Carrying out four successive years of a variety-N-disease multifactorial trial using 20 varieties at three N rates to investigate the impact of disease pressure on yield and NUE and nutrient acquisition, and the impact of canopy development on disease susceptibility.

In 2019, the treatment structure of the WGIN Diversity trial was changed. The number of varieties grown was reduced from 30 to 20, the number of nitrogen (N) treatments was reduced to three (100, 200 and 350 kg N/ha), dropping the zero N treatment. However, as well as growing all varieties and N treatments with standard farm practice pesticide inputs, a new treatment, with reduced pesticide inputs was introduced, doubling the size of the experiment, and was fully incorporated in the statistical design. This reduced input treatment was included to investigate N by variety by pest and disease interactions. It also provides the opportunity to investigate the identification of pest and disease issues by remote sensing. In 2019 one low rate fungicide application was made at the T3 timing to the reduced input treatment to prevent disease completely destroying the worst affected plots in the experiment. No insecticides were applied. The experiment was sown on 9th October 2018 and harvested 1-2nd September 2019. As expected, grain yields with reduced agrochemical inputs were reduced (Figure 7), increasingly so with increasing applications of N fertilizer.

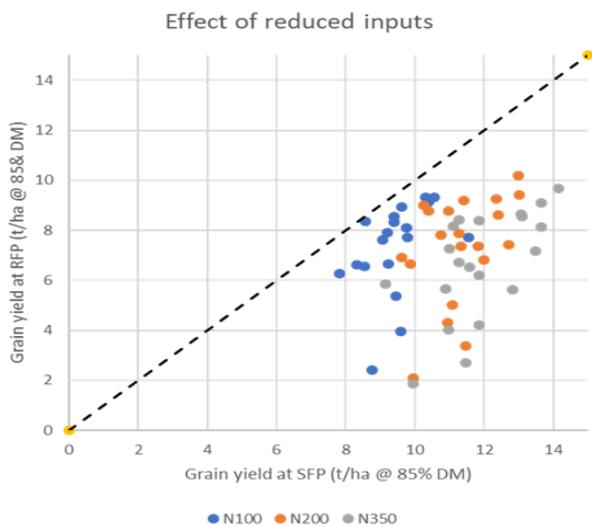


Figure 7 Grain yield with Standard Farm Practice (SFP) plotted against grain yield at Reduced farm practice (RFP) one half rate fungicide application and no insecticides.

The overall yields were higher in 2019 than 2018, and there was a greater response to N fertilizer. The highest yielding variety, Graham, yielded over 14t/ha with 350kg N/ha (Figure 8). The highest yielding varieties gave higher yields with 350 kg N than 200. At the lower N rate of 100kgN/ha, the variety Soissons gave a similar yield with RFP as SFP. Grain and straw samples were collected at harvest from all plots and have been analysed for major and minor nutrients. Data analysis is ongoing. The second experiment was drilled on 30th October 2019. Despite the wet autumn and winter the experiment established successfully.

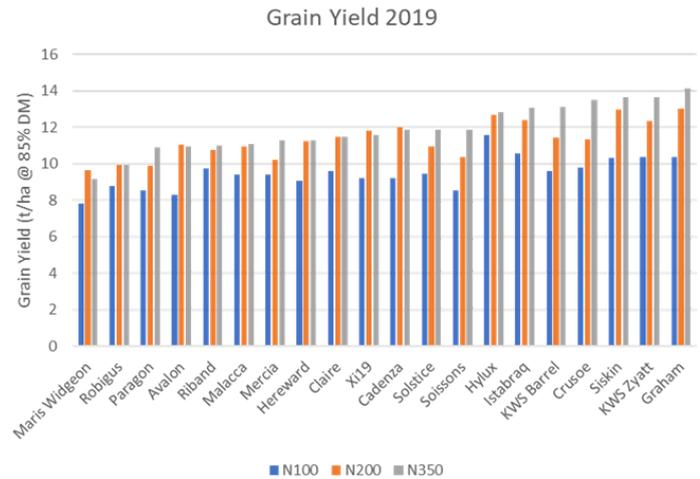


Figure 8 Grain yields of the 20 varieties grown in 2019, at standard farm practice rates of inputs.

- Developing remote sensing applications (using drones) to detect and quantify disease occurrence

The Diversity trial has already proved instrumental in developing UAV based phenotyping methodologies and is now being used to develop remote sensing capabilities for assessing disease occurrence, using the new reduced pesticide treatment described above. For the remote sensing of disease, two technologies have been used: high resolution imaging and hyperspectral reflectance. The hyperspectral reflectance is measured with a handheld device (Tec5) to identify spectral signatures associated with disease. The imaging has used RGB cameras, either handheld or UAV mounted. The UAV was flown at low altitude, 5m, to provide the greater resolution. Ground truthing in 2019 consisted of three traditional disease scoring campaigns, one for early foliar diseases, one to identify disease on the fully emerged flag leaf and one when the ear is fully emerged. Whilst quantifying canopy senescence can be achieved accurately, signatures for specific diseases have not been identified, as in 2019 one disease, yellow rust, predominated. Problems were also encountered

distinguishing general leaf necrosis and necrosis-associated with yellow rust pustules that had become prematurely brown due to frequent rain showers. In 2019, for the fourth year running, RRes UAV staff and equipment have been used to gather data from JIC WGIN trials.

- **Release of compiled yield and NUE data for all WGIN Diversity trials (2004-2019)**

The WGIN germplasm diversity trial is an example of a multi-variety, multi-N treatment series of trials conducted over multiple years. Data from the initial years (2004-2008) of these trials reported variation in yield and N-responses and contributing physiological processes (Barraclough et al. 2010; 2014). The trials have continued to the present date and have involved a large panel of modern commercial hexaploid wheats (varieties introduced between 1964 and 2016) and **data has been compiled for trials from 2004-2019** and is available from the WGIN homepage. In most years there were four N rates, from zero to 350 kg N/ha/yr, which represents no input through to excess applied N. All trials were conducted following local commercial agronomic practice, at the Rothamsted Farm in Hertfordshire in the UK. Whilst more than 60 varieties were examined in total, a smaller **subset of 15 core varieties have been grown for most years**. Data from all 16 years can be downloaded as one large excel file here: <https://rrescloud.rothamsted.ac.uk/index.php/s/714jNYDMY9rvUqL> or via the link on the WGIN homepage. Additionally, the **final report to defra** which includes a **detailed statistical evaluation** is available here:

<http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=19975&FromSearch=Y&Publisher=1&SearchText=CH0109&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description>

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)

- **Resistance and susceptibility to cereal aphids and BYDV in wheat (RRes)**

Cereal aphids and the diseases they transmit, including *Barley yellow dwarf virus* (BYDV), remain a significant consideration to many wheat growers and breeders. Concerns and speculations over the future impact of potentially increased levels of disease are fuelled by changes in pesticide regulations, primarily the recent neonicotinoid ban, and the reduced effectiveness of alternative pesticides. Both of the main cereal aphid pests known to spread BYDV, *Rhopalosiphum padi* and *Sitobion avenae*, have been shown to be developing resistance to pyrethroid pesticides, the main class of insecticides now used for aphid control after the neonicotinoid ban. See WGIN press release November 2018.

<https://www.rothamsted.ac.uk/news/future-wheat-harvests-very-vulnerable-disease-warn-experts>

We are monitoring for important aphid species and will also be looking at BYDV infection rates and the impacts of the virus across the 2019/20 Rothamsted WGIN diversity field trial (**Figure 9**). This will help to clarify current disease pressures across the range of wheat varieties included in the 2019/20 diversity panel and recommend potential strategies for controlling aphids and BYDV. We also intend to work with farmers and breeders who are happy for us to perform sampling in their fields. This would not affect wheat production in any way, we only need small samples from a few plants per field.



Figure 9 Plots of the WGIN wheat diversity field trial, taken early May, 2020.

Mapping populations from *Triticum monococcum* lines showing partial resistance to both *R. padi* and *S. avenae* have been developed after extensive phenotyping of ~1000 diverse wheat varieties. Going forward, these populations which have already been screened for aphid resistance through to the F₃ population will also be assessed for BYDV tolerance/resistance and transmission. The development of aphid and/or BYDV-resilient wheat varieties would provide a sustainable alternative to pesticides.

Recently, screening of 20 wheat varieties from the WGIN diversity trial for BYDV resistance has been performed through controlled exposure of young plants to BYDV followed by sampling and detection of BYDV using a new PCR-based assay. The results of this work are summarised in **Figure 10**. The level of BYDV infection was lower than expected, with the proportion of infected plants being between 0 and 0.3 for samples taken at the seedling stage and between 0 and 0.6 for flag leaf samples. Only the variety Solstice had no detectable BYDV in either leaf sample.

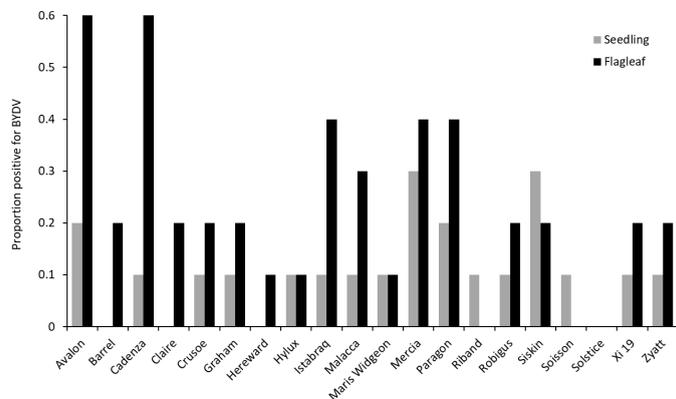


Figure 10 Susceptibility of 20 wheat varieties to BYDV infection from previous WGIN diversity trial at Rothamsted Research, Harpenden.

Most varieties had a higher proportion of BYDV plants infected at the flag leaf stage than the seedling stage. This was particularly evident for four varieties: Avalon, Cadenza, Istabraq and Paragon. Only the variety Siskin exhibited the reverse trend. Two varieties, Barrel and Hereward, had no infected plants detected at the seedling stage, whereas infected plants were detected at the flag leaf stage. Collectively, this data indicates that 19 of the 20 cultivars present in the WGIN 2019 Diversity trial could be considered susceptible to BYDV infection. Additional lines were suggested by stakeholders and

breeders for BYDV screening. These included 17 hexaploid wheat landraces, 33 synthetic wheat lines developed by NIAB EMR, and three commercially available cultivars (winter wheats Hereward and Solstice, and the spring wheat Paragon). At this time, only first leaf samples have been tested for BYDV. No individual wheat line demonstrated complete susceptibility across all plants challenged, including all commercial wheat germplasm (**Table 3**). A total of 19 wheat lines presented neither a clear indication of BYDV presence nor intermediate score (based on the new PCR-based assay) across any plants tested. These lines were considered to be uniformly resistant based on the samples processed and will be investigated further. A notable inclusion to the wheat lines considered to be potentially resistant was Solstice. Of the 33 Solstice plants challenged, a single plant was identified as having an intermediate reading for BYDV. None were identified as clearly containing BYDV. This is in stark contrast to Hereward and Paragon, the other two commercial wheat lines tested, with multiple plants of these cultivars identified as clearly containing BYDV.

Table 3 BYDV assay results of first leaf samples taken from commercial wheat cultivars one week after being challenged with BYDV-carrying *Rhopalosiphum padi*, and unchallenged control plants grown in parallel.

| Wheat cultivar | Number of <i>R. padi</i> /BYDV-challenged samples | | | Number of unchallenged control samples | | |
|----------------|---|---|---|--|---|---|
| | R | I | S | R | I | S |
| Hereward | 16 | 1 | 2 | 8 | | 1 |
| Paragon | 13 | 5 | 3 | 7 | 1 | |
| Solstice | 32 | 1 | | 8 | | |

BYDV resistance, 'R', classed as any samples where assay threshold suggested no BYDV present; intermediate, 'I', where an intermediate reading was recorded; susceptible, 'S', where assay score clearly indicated the presence of BYDV. Where no value presented, 0 samples identified. Wheat cv. Solstice where no clear indication of BYDV was identified in challenged and unchallenged plants highlighted (grey).

A small number of live aphids were observed during wheat harvesting after insecticide treatments, perhaps explaining why some unchallenged control samples were also identified as containing BYDV. This highlights that, even within a controlled environment, insecticide treatments cannot be considered absolutely effective in controlling the spread of aphids and BYDV.

For further information on this aspect of the WGIN project contact Lawrence Bramham at lawrence.bramham@rothamsted.ac.uk.

Foliar Disease (RRes)

- Replicated field experiments of lines carrying different mutant alleles of the recessive *mlo* resistance gene assessed for disease susceptibility, growth and development

In barley and tomato, the atypical recessive resistance gene *mlo* confers broad spectrum resistance to powdery mildew and other pathogens (reviewed (Acevedo-Garcia et al (2017) Plant Biotechnology Journal 15, 367-378) and is now used commercially in European breeding programmes. Recently, the equivalent mutant has been generated in hexaploid wheat by screening the WGIN 1 TILLING population to identify mutations in the three homoeologous genes and combine these through conventional breeding. In seedling tests, high levels of resistance to powdery mildew were identified in the triple mutant and some double mutant lines (Acevedo-Garcia et al (2017). In WGIN4, we are field testing the triple mutants and some double mutants for resistance to various fungal pathogens as well as Yellow rust and Fusarium. In this context, it is also noteworthy that the best Yellow rust resistant line 4 (57-1-13), gives neither Fusarium control nor increased Fusarium susceptibility. The new *mlo* yellow rust trial was sown on the Rothamsted Farm in March 2020 and again natural yellow rust infections were the predominant disease. These two *mlo* field trials revealed that 5 triple mutant lines (from a total of 13 lines tested) showed a small but consistent reduction in yellow rust levels from mid-May onwards in leaf layers 4, 3, 2 and 1 (flag leaf) (**Figure 11**).

One of the *mlo* field trials in 2019 (19/R/WW/1926 Trial 2) was also spray-inoculated with *Fusarium graminearum* spores at anthesis (GS61). The ears of each plot were bagged for 24 hours to promote infection. At 21 days post-inoculation 20 ears per plot were scored for Fusarium Head Blight (**Figure 12**). No obvious relationship was observed between the levels of yellow rust on each line and subsequent Fusarium infection levels. In terms of trade-offs between the resistance conferred by *mlo* against multiple diseases, line4 (52.3.08) provides moderate control of both

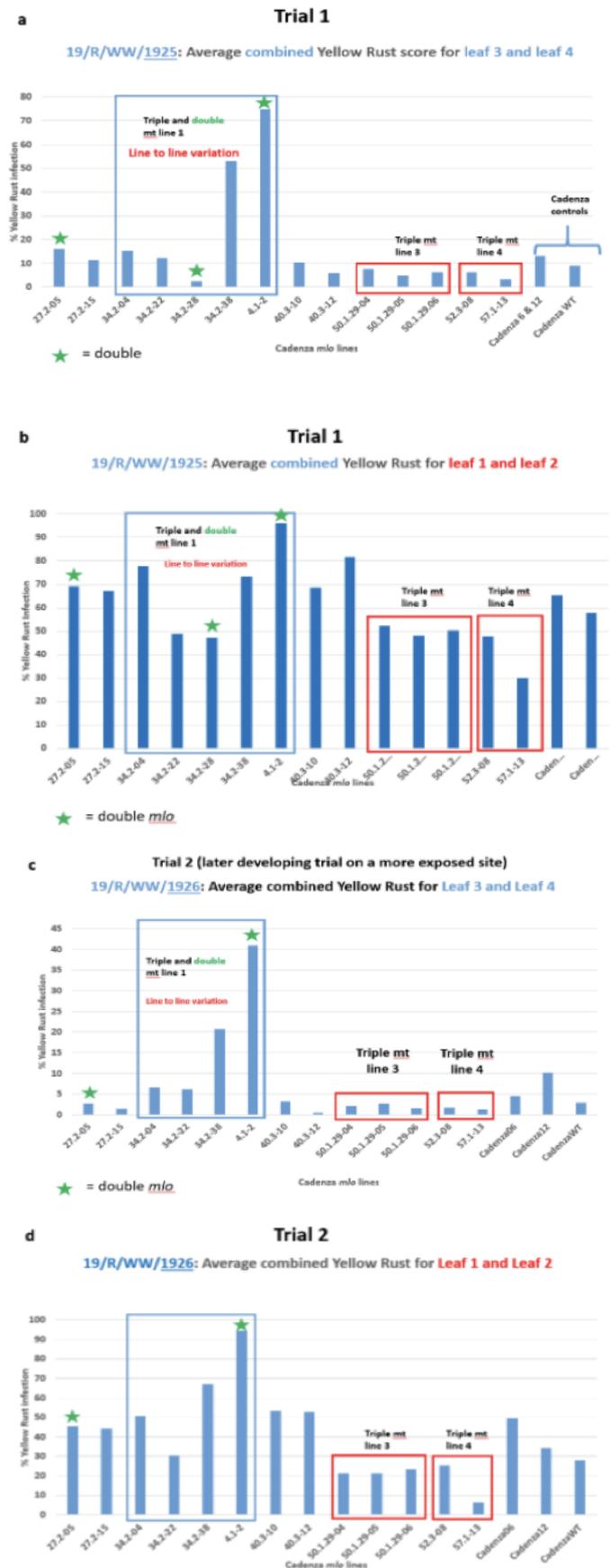


Figure 11 Yellow rust disease assessment scores for field experiments 19/R/WW/1925 = Trial 1 (a, b) and 19/R/WW/1926 = Trial 2 (c, d). Some of the *mlo* triple mutants displaying a reduction in infection to yellow rust are outlined in a red box when compared to the wildtype Cadenza parents (Cadenza 6 and 12).

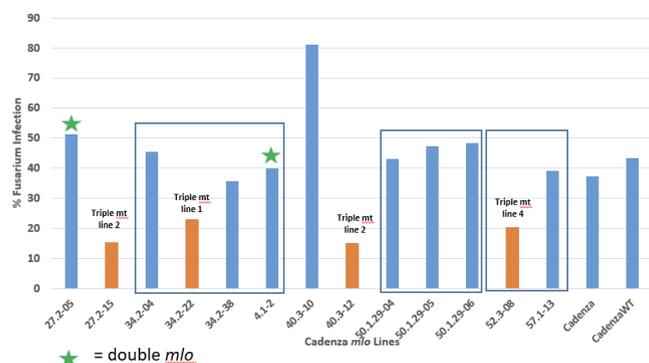


Figure 12. Fusarium Head Blight infection for field trial 19/R/WW/1926. Some of the *mlo* mutants displayed a reduction in *Fusarium graminearum* infection are shown as orange bars when compared to wildtype Cadenza.

For further information on this aspect of the WGIN project contact Gail Ganning (gail.canning@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 2019 Newsletter, our third crossing strategy used for crossing *Triticum monococcum* into hexaploid wheat has been successful. As a reminder, this strategy, using *T.durum* as a bridging species, is shown again in **Figure 13**. Potential **F₁ hybrid** grains would be **A^mAB triploid** containing parts of the *T.monococcum* **A^m** chromosomes but also most likely being **male sterile**. The resulting '**F₁ complex**' is thought to be **pentaploid**, missing one Paragon D genome and substituting one Paragon A genome for one *Tm* **A^m** genome or (more likely) segments of A^m chromosomes within the Paragon A genome. Subsequent backcrosses (BC₂F₁ to BC₁F₁) would eventually generate the desired hexaploid hybrid **A^mABBDD**, with fertility increasing in each backcross generation. Initially, F₁ complex plants could only be produced with MDR031, which has strong take-all resistance. Therefore, at the same time as backcrossing the five MDR031 F₁ complex plants with Paragon, a second round of crosses was started for MDR049 (aphid resistance) and MDR308 (Septoria resistance). Where included in figures / tables in this document, generated plants and grain include '**R2**' in

their names. Thus, introgression for MDR049 and MDR308 is one stage behind MDR031.

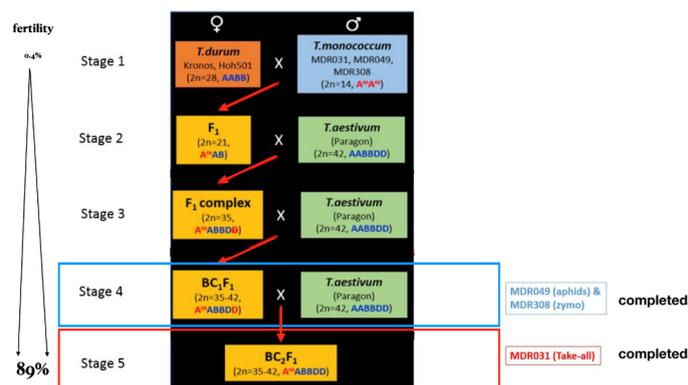


Figure 13 A reminder of the introgression crossing strategy used during WGIN 3 & 4, including current state of backcrossing for the three MDR lines.

• Current State of (Back)Crossing

For R2, **13 F₁ hybrid grains for MDR049** and **90 F₁ hybrid grains for MDR308** were generated. From these, again a very limited number of F₁ complex plants were generated. **Table 4** lists the number of F₁ complex plants (F₁C) generated for all three *T. monococcum* accessions.

Table 4 Total number of F₁Complex plants generated for the three Tm accessions MDR031, MDR049 and MDR308. All backcross (BC) plants generated subsequently derive from these nine F₁C plants.

| Tm parent | no. of F ₁ C plants | names of F ₁ C plants |
|-----------|--------------------------------|----------------------------------|
| MDR031 | 5 | F1C#24 |
| | | F1C#47-1 |
| | | F1C#86-1 |
| | | F1C#86-2 |
| | | F1C#94 |
| MDR049 | 1 | R2#2-7 |
| MDR308 | 3 | R2#13-1-1 |
| | | R2#13-1-2 |
| | | R2#14-1-1 |

The current state of backcrossing is summarised in **Table 5**, showing the number of grains generated at BC₁, BC₂ and BC₃ stages as well as the number of individual plants used to generate the next stage (e.g. BC₁ plants generate BC₂ grains, etc). A large number of grains were generated for all three Tm accessions at the latest (current) backcrossing stage. At all BC stages, selfed grain from all plants was also harvested, because if the BC plant has managed to retain the

introgressed segment(s), these selfed plants would be expected to be homozygous also for the Tm segments. This means that if resistance against any of the target pests and pathogens has indeed been retained in these BC plants, this should be stronger in the selfed plants. Several rounds of backcrossing with the hexaploid Paragon serve to increase the stability of the hexaploid genome of the introgressed plants while at the same time diluting the portions of *T. monococcum* introgressed segments. We have therefore halted the backcrossing at the BC₃ stage to establish first which of these plants show the desired resistance, and carry those plants forward through selfing using single seed descent (SSD), to make certain that the introgressed segments are homozygous.

Table 5 Number of backcross grains (BC#) generated and numbers of BC plants used to generate the next stage of BC grain. BC1 plants → BC2 grain, BC2 plants → BC3 grains. REV denotes reverse crosses where Paragon was used as the female and a BC1 plant of F1C#47-1 as the male (Paragon (♀) x BC1 - [F1C#47-1] (♂))

| Tm parent | F1C plant | BC1 grains | BC1 plants | BC2 grains | BC2 plants | BC3 grains |
|-----------|-----------|------------|------------|------------|------------|------------|
| MDR031 | F1C#24 | 15 | 5 | 213 | 40 | 534 |
| | F1C#47-1 | 6 | 2 | 203 | 16 | 196 |
| | F1C#86-1 | 79 | 8 | 475 | 36 | 1004 |
| | F1C#86-2 | 18 | 2 | 101 | 5 | 187 |
| | F1C#94 | 55 | 8 | 406 | 56 | 1400 |
| MDR049 | R2#2-7 | 122 | 10 | 328 | | |
| MDR308 | R2#13-1-1 | 37 | 3 | 153 | | |
| | R2#13-1-2 | 52 | 12 | 426 | | |
| | R2#14-1-1 | 36 | 8 | 392 | | |
| MDR031 | REV-X57 | 21 | 5 | 64 | | |
| | REV-X58 | 21 | 5 | 115 | | |

● **Resistance Trials under glasshouse and field conditions**

So far, only one Septoria infection test has been carried out in the glasshouse, under the guidance and help of Dr. Kostya Kanyuka (RRes). **Figure 14** shows the encouraging results from this test. The BC₂ plants shown here were grown from BC₂ grains derived from only 4 crosses - ie X94, X134, X161 and X166 - and individual plants from each cross show varying levels of spore reduction. This again highlights the point that each grain produced on the same ear represents a unique crossing event with differing degrees of introgression. This first infection test is encouraging in that some BC₂ plants show highly reduced Septoria susceptibility, especially **BC₂-X134-4** and **BC₂-166-2** with >10 fold reduction (**Figure 14**).

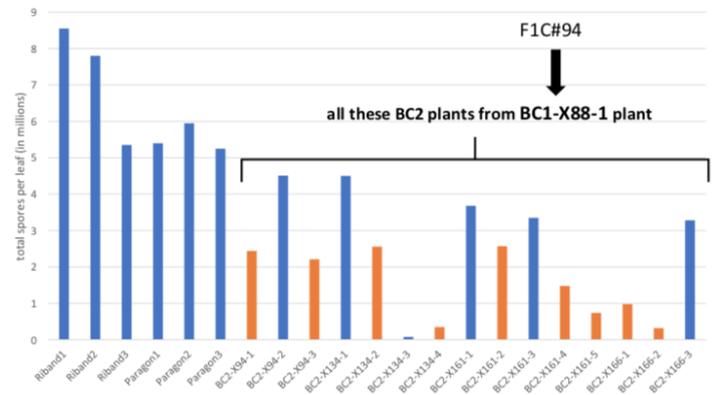


Figure 14 First *Septoria* resistance tests on seedling *T. monococcum* introgressed plants.

Riband and Paragon (the male donor for the Tm Introgression crosses) are highly susceptible hexaploid wheat varieties. Spore counts here range between 5.2 to 8.5 million spores total per inoculated leaf. All BC₂ plants shown here are descendants of F1C#94. orange bars = plants with highly reduced spore counts compared to the susceptible controls. Although BC₂-X134-3 has the lowest spore count, this is because the leaf had died (detached from the plant).

We applied successfully to the Business Continuity Panel at Rothamsted (created due to Covid 19) to be allowed to plant a field trial with some of the selfed grain in May 2020 during complete lockdown. This trial would determine whether any of the lines generated and tested show resistance to yellow rust, brown rust or powdery mildew as well as the specific resistances we are intending to introduce, namely Take-all, Septoria, Aphids under field conditions with no fungicide/ insecticide applications. The individual introgressed lines to be used in this first *Triticum monococcum* Introgression field trial as well as their pedigree are shown in **Table 6**. The next newsletter will contain details of this very promising trial.

Table 6 Introgressed lines selected for the 2020 Field Trial. Lines with the same colour originate from the same F1 complex plants. There are five F₁C derived lines for MDR031 (Take-all) introgression, two for MDR308 (Septoria) and one for MDR049 (aphids).

| Design # | ALL SELFS (S-#) | F1hybrid | F1C | BC1 | BC2 |
|----------|-----------------|----------|---------|---------|---------|
| 1 | BC1-X12-1 | Kronos | Fielder | | |
| 2 | BC1-X12-3 | | | | |
| 3 | BC1-X87-1 | | | | |
| 4 | BC1-X173-1 | | | | |
| 5 | BC1-X27-1 | | | | |
| 6 | BC1-X147-1 | | | | |
| 7 | BC1-X75-1 | | | | |
| 8 | BC1-X88-1 | MDR031 | | Paragon | Paragon |
| 9 | BC1-X122-1 | | | | |
| 10 | BC1-X161-3 | | | | |
| 11 | BC1-X186-2 | | | | |
| 12 | BC1-X187-1 | | | | |
| 13 | BC1-X187-2 | | | | |
| 14 | BC1-X187-3 | | | | |
| 15 | BC1-X74-1 | | | | |
| 16 | BC1-X115-1 | | | | |
| 17 | BC1-X189-2 | | | | |
| 18 | BC1-X224-1 | Hoh501 | | | |
| 19 | BC1-X224-2 | | | | |
| 20 | R2#2-7 | | | | |
| 21 | R2#13-1-1 | MDR049 | | | |
| 22 | R2#13-1-2 | | | | |
| 23 | R2#16-7 | MDR308 | | | |
| 24 | BC2-X65-1 | | | | |
| 25 | BC2-X113-1 | MDR031 | | | |
| 26 | BC2-X113-3 | | | | |
| 27 | BC2-X150-1 | | | | |
| 28 | BC2-X150-2 | | | | |
| 29 | BC2-X150-3 | | | | |

● **Extent of Introgression Events in Introgressed Lines**

The **35k Axiom Breeders' Array** (University of Bristol) was used to obtain molecular genetic evidence for *T. monococcum* introgression in the F1C complex plants. DNA from the parent and hybrid plants (at this time only for MDR031) was hybridised to over 35000 markers at Bristol and each marker scored for homozygosity (AA or BB) and heterozygosity (AB). If both MDR031 and [Paragon/Kronos] are **homozygous but opposite** (MDR031= AA, Par = BB, or reversed), any Axiom marker giving a **heterozygous call (AB) for the hybrid plants indicates an introgression event**. This is also true if the Tm parent is heterozygous. It is important to note that this method only allows scoring of heterozygous markers, and that the actual number of introgression events is likely to be considerably higher, because alleles where *T. monococcum* and the tetraploid/ hexaploid parents carry the same SNPs cannot be revealed with this method but will require genome sequencing of the parent and introgressed lines.

Analysis for hetMarkers in the five F₁Complex plants and some BC₁, BC₁-Selfed and F₁C-Selfed has been completed. **Figure 15** shows the data for all three sub-genomes to determine preferential introgression in the three sub-genomes. While this indicates that the majority of introgression events occurred in the A genome as expected (because Tm has only a distant relative of the A genome, but no B or D genomes) there is still significant introgression in the B and D genomes. Notably, chromosome 4B shows more introgression events than Chr4A and Chr4D (**Figure 15a**) while overall introgression ranks:

A genome>>>B genome>>>D genome.

Figure 15b shows one chromosome where the preference for introgression into the A homoeologue is more pronounced still. This may prove to be important, because there is evidence from the literature that *T. monococcum* resistance genes reside preferentially on Chr7A^m.

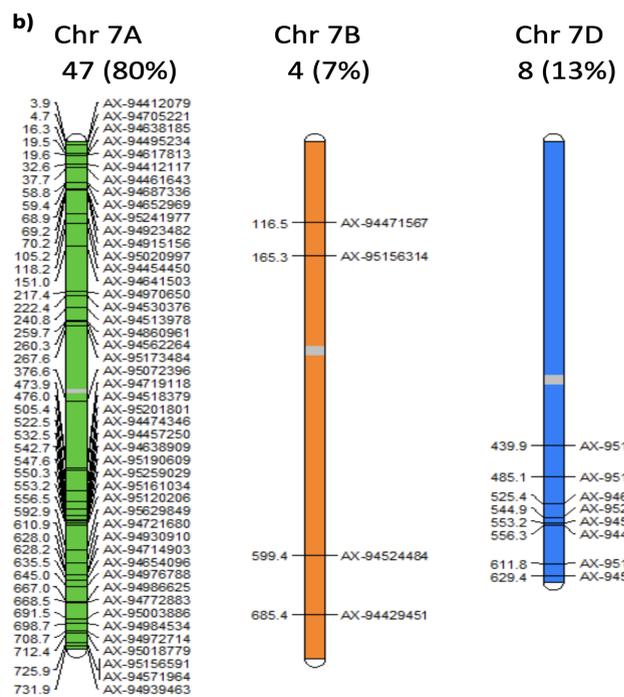
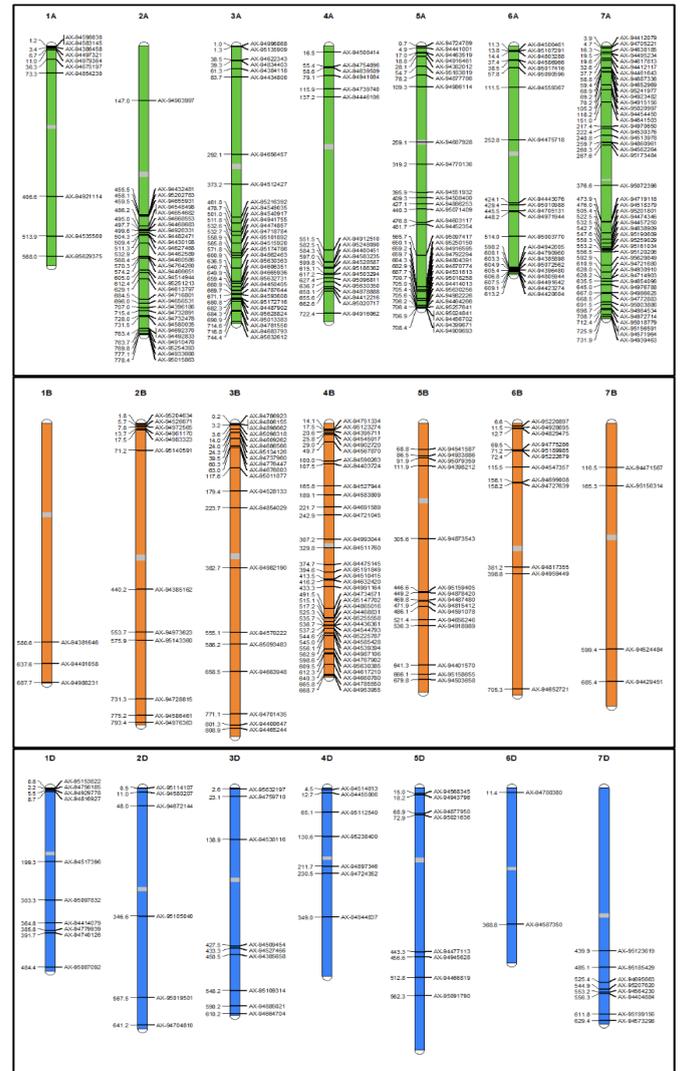


Figure 15: Location of hetMarkers showing Tm introgression events in the whole wheat genome (A=green, B=orange, D=blue) for the five MDR031 F₁C plants combined and (b) the details for chromosome 7.

Finally, **Figure 16** shows the completed hetMarker analysis of the A genome for the five MDR031 derived F₁C plants. While there are numerous introgression events, all five F₁C plants differ in frequency and location of hetMarkers, but also share some events. The density of hetMarkers differs considerably from chromosome to chromosome for each F₁C plant, but also between F₁C plants. For example, for all five F₁C plants, there are very few hetMarkers on Chr1A while the highest numbers occur on Chr5A. This analysis also clearly shows the segregating nature of these introgression events - the two F₁C plants **F₁C#86-1** and **F₁C#86-2** (blue and pink markers in Figure 16) derive from crosses on the same F1 hybrid ear but show mostly differing introgression events on all chromosomes apart from chromosome 5A. These two plants are also phenotypically very different, at least with respect to plant height.

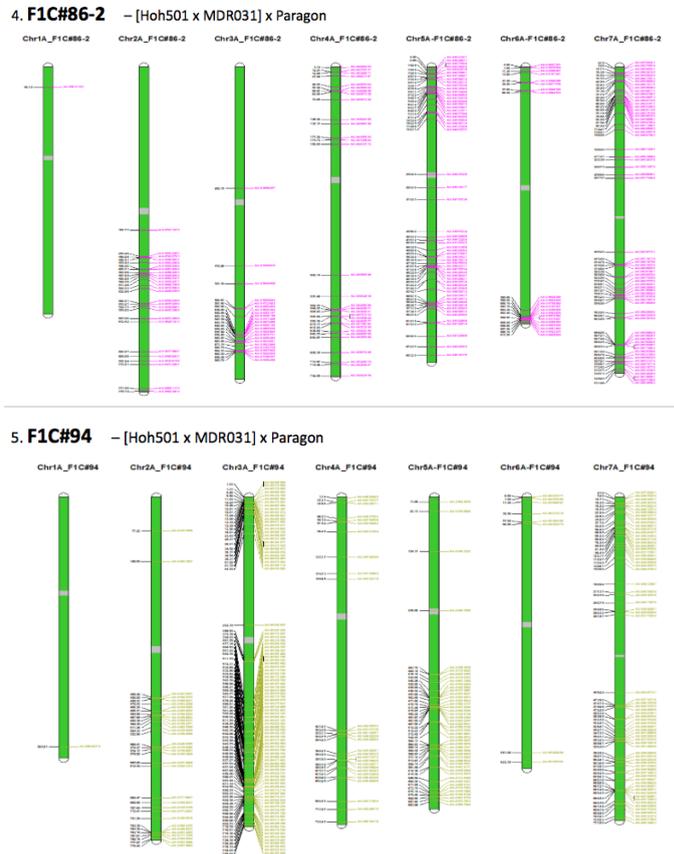
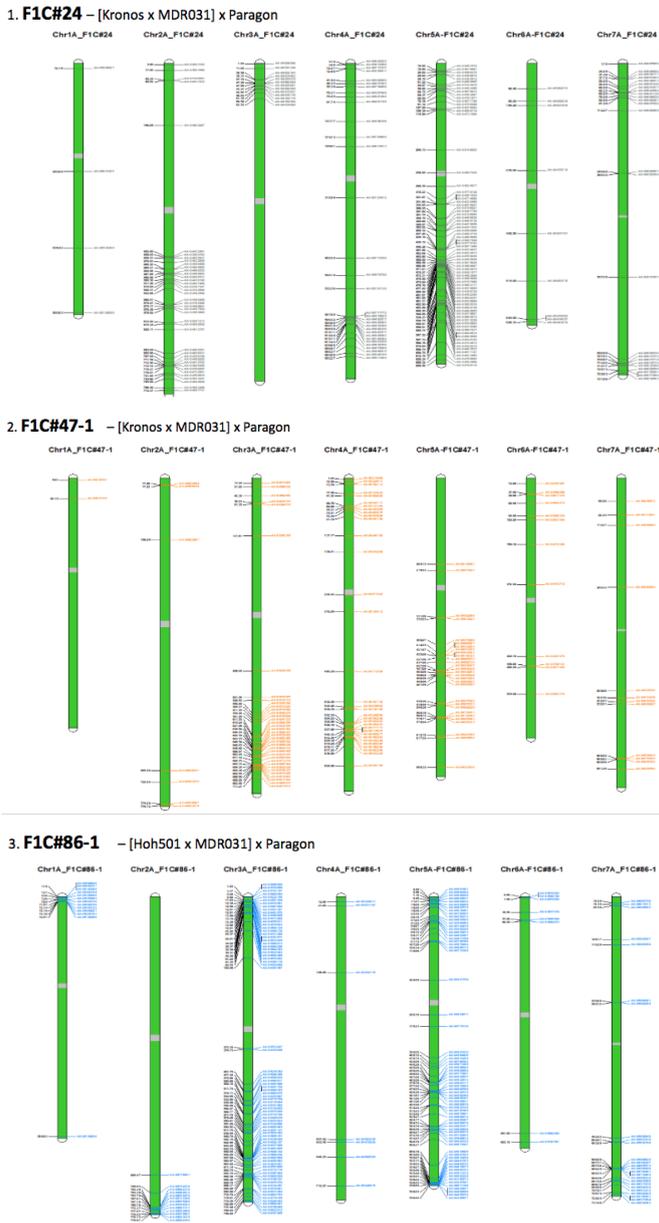


Figure 16: Location of hetMarkers in the A genome of all five hybrid F₁C plants for MDR031 Introgression. For each F₁C plant, the 7 chromosomes are arranged from left to right and can be directly compared between the five plants with regards to number of hetMarkers and their location.

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

Section 3 Events

- An overview presentation on the WGIN project was given at **CropTech, Peterborough in Nov 2019**. This presentation will be available shortly from the WGIN website.
- The **2020 WGIN Stakeholders' Meeting** was postponed and held as a virtual event on 3rd March 2021 due to Covid19. The programme and all the presentations are available as usual from the WGIN website. This year the topic for the Panel Discussion was **"Gene Editing and Innovative Breeding Opportunities for Wheat"**. Introductory presentations were given by **Huw Jones** (Aberystwyth University) and **Emma Wallington** (NIAB). The panel discussion members were **Johnathan Napier** (RRs), **Emma Wallington** (NIAB), **Tom Allen Stevens** (Farmer, editor of CPM magazine) and **Anthony Keeling** (Director,

Elsoms UK). And as it happened, **Johnathan Jones** (The Sainsbury Laboratory, Norwich) joined the panel for most of the discussion. **Peter Shewry** (RRes), our longstanding WGIN chairman, guided the discussion throughout. As a lot of interesting questions were raised, you can **listen to the whole discussion on the WGIN homepage** (www.wgin.org.uk).

- A selection of WGIN experiments, trait discoveries and new technologies will be on display at the annual **Cereals event in Lincolnshire (30th June-1st July, 2021)** at the Rothamsted Research Exhibit.
- The **2021 WGIN annual Stakeholders' Meeting** will take place sometime in **November 2021** at Rothamsted Research. Hopefully this can be a real on-site event again, but we will make a decision closer to the time. We have yet to decide the topic for this year's panel discussion. If you are interested in suggesting a topic or becoming a panellist please contact Peter Shewry (peter.shewry@rothamsted.ac.uk) or Mike Hammond-Kosack (wgin.defra@rothamsted.ac.uk).

Section 4 News

- 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, we have also featured different WGIN article published sometime in the past 5 years.
- The next WGIN Newsletter will be available in December 2021 and will provide further updates on the WGIN experiments and analyses done during 2020 and 2021.
- **New arrivals on the WGIN project**
 - **Dr Javier Palma-Guerrero** joined Rothamsted Research in January 2020 from the ETH Institute in Switzerland to lead the take-all root disease research activities, carrying on the work of Dr. Vanessa McMillan who has now joined NIAB.

- **Dr Lawrence Bramham** joined Rothamsted Research in February 2020 from the Wellesbourne Campus, University of Warwick to focus on aphid insect pest control as well as the virus species transmitted by aphids, carrying on the work of Dr. Gia Aradottir who has now joined NIAB.

- **Interconnecting the four Defra supported GINs:** Defra continues to support long-term Genetic Improvement Networks (GINs) to improve major UK crop varieties. Since 2018 the four GINs - OREGIN, PCGIN, VEGIN and WGIN - have been brought together under the Crop Genetic Improvement Platform. This common governance structure encourages cross fertilisation of ideas and develops shared understanding of new approaches and technologies. We also try to ensure our annual stakeholder meeting days don't clash! A joint GINS website established in 2020 is available at <https://defracropgenetics.org>. Stakeholders can obtain for each GIN information on their latest publications and planned outreach events.

The WGIN Management would like to thank Vanessa and Gia for their many years of productive involvement within the WGIN project and wish them all the best in their new positions.

Section 5 Publications

- Crop Production Magazine – pdf on WGIN website - August 2020
- Hawkesford MJ, Riche AB (2020) Impacts of G x E x M on Nitrogen Use Efficiency in Wheat and Future Prospects. *Frontiers in Plant Science*, **11**, 1157. (doi: [10.3389/fpls.2020.01157](https://doi.org/10.3389/fpls.2020.01157))
- An evaluation of recent trends in nitrogen use efficiency of UK wheat. (2020) DEFRA report EVID 4 CH0109
<http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=19975&FromSearch=Y&Publisher=1&SearchText=CH0109&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description>
- AHDB Science report No 50
<https://ahdb.org.uk/identification-of-bydv-resistance-in-wheat> (published May 2020)

- Alba Farre Martinez, Clare Lister, Sue Freeman, Jun Ma, Simon Berry, Luzie Wingen and Simon Griffiths (2021) Resolving a QTL complex for height, heading, and grain yield on chromosome 3A in bread wheat (2021) *Journal of Experimental Botany* **72**, 2965–2978
<https://doi.org/10.1093/jxb/erab058> (published Feb 2021)
- Zhou H*, Riche AB, Hawkesford MJ, Whalley WR, Atkinson BS, Sturrock CJ, Mooney SJ* (2021) Determination of wheat spike and spikelet architecture and grain traits using X-ray Computed Tomography imaging. *Plant Methods* (doi: 10.21203/rs.3.rs-101191/v1).
- Palma-Guerrero, J., Chancellor, T., Spong, J., Canning, G., Hammond, J., McMillan V. E. and Hammond-Kosack, K.E. (2021) Take-all disease: New insights into an important wheat root pathogen. *Trends in Plant Sciences*
<https://doi.org/10.1016/j.tplants.2021.02.009> (published March 2021).

Section 6 PhD projects ongoing using WGIN genetic materials, field trials and /or data

- Industrial year student **Sarah Bailey**, from the University of Nottingham, on a project with the Avalon x Cadenza NILs and the CSSL lines which were generated from these resources.
- BBSRC-DTP student (University of East Anglia Oct 2018-Sept Sept 2022). The project is entitled 'The hunt for healthier wheat starch' Main Supervisor Brittany Hazard, Norwich.
- **Tania Chancellor**, BBSRC - University of Nottingham DTP (Oct 2017- Sept 2021) rotation project Feb-March 2018, main PhD project started April 2018 'Evaluating the potential of beneficial Gaeumannomyces species for the control of take all disease in wheat". Supervisors: Javier Palma-Guerrero, Kim Hammond-Kosack, Smita Kurup, academic supervisor Matthew Dickinson
- **Henry Tidd** - BBSRC University of Nottingham iCASE DTP (Oct 2018-Sept Sept 2022) 'Characterisation of major genes (Stb)-mediated resistance to Septoria

tritici blotch disease in wheat'. Supervisory team: Kostya Kanyuka (RRes), Ruth Bryant (RAGT Seeds), Rumiana Ray (University of Nottingham).

- Project on Nutrient use efficiency, sponsored Feb 2018 - Jan 2022, fully funded by Roullier, a French agrochemical company, Supervisor Malcolm Hawkesford (RRes)

Section 7 Scientific Outreach Activities

- **Virtual Cereals 2020** (June) WGIN webinar given by Kim Hammond-Kosack >200 attendees
- New **Take-all root disease video** prepared for the virtual display at Cereals June 2020 - available on YouTube <https://www.youtube.com/watch?v=JcVlx7R5QZl> and from the WGIN website - Participants: Javier Palma-Guerrero, Tania Chancellor, Jess Spong, Jess Hammond, Gail Canning and Kim Hammond-Kosack. Video and root photography by Mike Hammond-Kosack and compilation by the visual communication team at Rothamsted Research
- WGIN activities included in a **CPM magazine article** on 'The Global Reach of Plant Sciences' July 2020 – Kim Hammond-Kosack and Simon Griffiths
- Rothamsted (Andrew Riche, Kim Hammond-Kosack, Alison Lovegrove) hosted the visit of Tom Allen-Stevens (editor CPM magazine) 13th August 2020 + gave a field trial visit.
- Simon Griffiths interacted with Tom Allen-Stevens and the Bofin group (www.bofin.org.uk) to encouraging farmers interested in doing **on-farm trials** to explore the efficacy of one Watkin wheat line which has shown good resistance to slugs (Sept 2020). Sufficient seed (50Kg) will be multiplied to take this forward.
- Virtual WGIN annual stakeholder event - 3rd March 2020 – This event attracted 76 attendees (25% of which were farmers / farm managers and 20% from commercial wheat breeding companies). The event was BASIS points registered. The invited five person panel discussion was on " Gene editing and innovative breeding opportunities for wheat " to coincide with the active UK government open consultation on Gene Editing. A video recording of the panel discussion and

copies of the meeting presentations are available from the WGIN homepage.

- CPM Magazine article on WGIN project, the stakeholder event and the GE debate in the Innovation Wheat Genetics section entitled - 'Hope for the wheats of wonder' (March *extra* issue 2021) contributors – Andrew Riche, Malcolm Hawkesford, Clare Lister, Simon Griffiths and Kim Hammond-Kosack. The CPM Magazine is read each month by around 10,000 UK arable farmers and agronomists.
<http://www.cpm-magazine.co.uk/wp-content/uploads/2021/03/CPM-March-Extra-2021.pdf>

- Two **take-all posters** prepared and distributed by email to Anglian Water and ~25 farms in a water

catchment region in Northamptonshire (February 2021). Posters prepared by Javier Palma-Guerrero, Gail Canning and Kim Hammond-Kosack. This is in preparation for on farm talks to devise a new collaborative project to use wheat genetics to protect water courses from farm run-off.

- Take-all press release '**Taking on Take all**' on the Rothamsted website to accompany the new Take-all review published in Trends in Plant Sciences (21st March 2021) - Javier Palma-Guerrero, Gail Canning and Kim Hammond-Kosack

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, Malcolm Hawkesford, Lawrence Bramham, Gail Canning, Javier Palma-Guerrero, Michael Hammond-Kosack (RRes); Clare Lister and Simon Griffiths (JIC).

