WGIN 4 Management Meeting
4th October 2018 @ Limagrain (Woolpit)

This was the first Management Meeting of the **DEFRA** funded **WGIN4**.

Minutes

Attendees:
Peter Shewry (PS) (**chair**), Andrew Riche* (AR), Malcolm Hawkesford (MH), Kim Hammond-Kosack*(KHK), Michael Hammond-Kosack* (MHK), Vanessa McMillan* (VM) (RRes), Clare Lister* (CL) (JIC), Ruth Bryant (RB) (RAGT), Nick Bird (NB) (KWS), Stephen Smith (SS) (Elsoms), Lucy James (LJ) (ADAS), Martin Cannell (MC) (Defra),
[*=gave oral presentation]

Apologies:
Matt Kerton (DSV-UK), Sarah Holdgate (NIAB), Simon Berry, Ed Flatman (Limagrain) and Dhan Bhandari (DB) (AHDB), Simon Griffiths (SG)

A. Welcome – Peter Shewry

1. Review of minutes from 1st February 2018 & June 28th (KHK)

- CL to confer with SG to finish the list of available mapping populations with the aim of putting this list on the WGIN website. CL suggested preparing a poster highlighting the availability of these for the WGIN SM on November 16th.
- AR/ MH suggested to delete the action item pertaining to the Diversity trial from the February minutes (agreed by all)
- MHK to produce a graph with Stakeholder meetings attendance shortly [nb: this was done on Oct 9th]
- 'seasonable lodging' (mentioned in a comment by SG on June 28th) to be changed to 'very variable lodging'

Nb: both minutes now approved and are on the WGIN website [http://www.wgin.org.uk/information/meetings.php]

B. Presentations:

2. Tools, resources, genotyping and phenotyping – (CL)

Details in presentation online (**pp1-18**).

Another Paragon x Garcia drought trial will be drilled this autumn. This was not planned but is necessary because of the highly variable conditions during the three drought trials during WGIN3.

Genotyping of the CSSL lines is ongoing. It has been completed for chromosomes 1, 2 and 3, but not yet started for chromosomes 4-7. Once completed these data will go on the WGIN website.

There are 10 WGIN 4 projects (**p6**) of which Clare focussed on (1) drought tolerance, (2) anchorage & lodging) and (3) Promotome analysis of flowering time genes (from Trait 7 and 8 lists).

CL has expanded the list of lines to be included in the drought/lodging panel. Currently there are ~120 lines in the first year trial, including the 20 lines from the 2018/19 Diversity trial (**p8**). There are only 3 Watkins lines (W110, W126 & W127) included.

Q. MH – are any Par x Garcia lines included?
A. CL – no, but will include some (nb: the 15 lines that performed best under the 2017 drought conditions)

Regarding 'Anchorage', CL asked for some advice and thought about how to best measure anchorage strength.

A summer student, Scott Sheldrick, analysed flowering time genes (from the Trait 7 and Trait 8 lists, in the promotome capture experiment) (**p10-17**), with regards to number of haplotypes (referred to as alleles in the presentation). Importantly, he also designed and tested some KASP markers based on the promoter SNPs which showed very good correlations. In addition, he succeeded in showing that observed variation in promoter genotypes is associated with phenotypic variation in height and flowering time for several genes, namely: FT4 (2B), FT4 (2D), TOE (1A), CO2 (6A), AGL10 (2B), AGLG1 (5A) and CO7 (6B). (**p17**).

Importantly, CL mentioned an example of a gene from another project, related to a gene in the Trait 7 list, where the promoter showed some coverage despite the fact that this gene had not been included in the WGIN Wheat Promotome Capture and **no MYbaits** had been designed for this promoter. This suggested mis-binding of MYbaits, and could be viewed as either a complication or added value.
C. MHK – has observed very similar coverage for homoeologue promoters which were only discovered subsequently, ie after the experiment had been completed. MHK was also initially worried about these additional results, but now is not and he has included an explanation in his presentation.

Nb: Kostya Kanyuka has also observed coverage for several *Stb* paralogues residing in a cluster with the cloned Septoria tritici disease resistance gene *Stb6* (the only *Stb* gene for which MYbaits were designed) (KK personal communication).

3. Update on WGIN Diversity Trial 2017 (AR)

Details in presentation online (**pp19-31**).

Soil Nitrogen was very high in 2018. This was because of a blackgrass infestation in the previous oats crop. The crop was cut early when still green meaning that some of the applied N was not used by the plants and thus remained in the soil. The 2017-2018 growing year also produced the lowest yields of ALL the Diversity trials years and with no beneficial N effect above 100kg/ha. An infestation of midges may also have contributed to the low yields in addition to the higher summer temperatures and lower summer rainfall.

For the 2019 Diversity trial the number of varieties has been reduced to 20 but the majority of core lines have been kept in and Claire and Robigus have been reintroduced after discussion at the last MM. This means that all 20 lines for this trial were also present in the 2018 trial.

C. MH – this will be a duplicate trial with fungicide treated and untreated/minimal treatment plots for all cultivars. Furthermore, the N=0 has been abandoned.

Q. KHK – Hereford has now been abandoned, despite being very high yielding. Do the breeders know what makes Hereford high yielding?
A. SS – it is a parent of many lines, high tillering with small ears but is very susceptible to brown rust which is why it didn't make the RL (recommended list).

The 2019 trial will involve disease scoring and we are going to explore whether disease symptoms can be detected using cameras on the UAV. So far most of the drone work has been done from a height of 50m, which involves taking lots of images and stitching them together which is very time consuming. Higher resolution required to detect disease symptoms implies a (much) lower flight height, but this generates considerable downdraft. The heights considered at present are either 3m or 5m which would increase the possible resolution from 1pixel = 1cm to 1pixel = 1mm. The downdraft could actually be advantageous to expose lower leaves to the camera. Also, initially only RGB images will be taken. There are 3 issues to consider: 1) taking a good image, 2) identifying where it was taken and 3) analysing what is contained in the image.

C. MH – we will end up with thousands of images per season. Manual identification of disease would require experts for each or this could possibly be automated. The capability exists at RRes but this has not been costed into WGIN4 and the capacity of high resolution pictures would be an issue.

C. KHK – a strong downdraft could blow aphids of plants and spread them further, potentially increasing insect damage and /or fungal disease levels.

4. Resistance to Foliar Diseases (VM)

Details in presentation online (**pp32-52**).

A Septoria resistance field trial has just finished which included 150 Watkins landraces, which had little or no Septoria in a 2008 trial, some CIMMYT lines from their Septoria Observation Nursery and 15 Wheat genotypes with known Stb resistance genes (KK) (**p34**). There are around 16 Septoria resistance genes but for quite a few of these it is not known how efficient they are in the field. For this trial only limited seed was available, which resulted in only 2 replicates per treatment. Avalon was drilled around the trial to encourage natural Septoria infection. A few issues with the trial included low natural disease pressure for Septoria in Hertfordshire, VM had discussed with Kostya Kanyuka whether it would be worth artificially inoculating the trial plots if the natural disease pressure remains low. Also, the low seed numbers resulted in low plant densities in most plots which also does not encourage Septoria disease build-up. The very dry spring and early summer further reduced disease impact because septoria relies on splash dispersal for infection. Next year the trial can be irrigated to encourage splash dispersal. But the biggest issue was how to control yellow rust. Bart Fraije (RRes) suggested a fungicide regime to selectively impact yellow rust, but this was not very efficient, probably because the T0 treatment was not applied.

However, the trial could be assessed for some of the genotypes. Some of the Watkins and CIMMYT lines showed strong resistance and the resistant Watkins lines have now been nominated for the WAK

Capture experiment. Of the known R gene containing lines, *Stb5* appears to be the most effective *R* gene (cultivar 'Synthetic 6X'). The others have various combinations of *R* genes, and *Stb6*, for example, works only against a small subset of septoria races. Half the lines could not be assessed because of 100% coverage of leaves with Yellow Rust.

Q. RB – are these Watkins lines the same that populations were created from?
A. VM – no populations have been developed yet for Septoria, only for Yellow Rust.

Q. RB – who recommended the CIMMYT lines?
A. VM – the Septoria Observation Nursery sends out a new set of 50 lines every year.

C. KHK – all the lines in the graph must also be resistant to yellow rust because they could be assessed for Septoria.

C./Q. PS – Who remembers David Royle (?) and his splashometer. Apparently the leaf angles were important for Septoria infection. Were leaf angles measured?
A. VM – No.

Regarding Yellow Rust resistance, five Watkins genotypes (W203, 231, 610, 733 & 786) with moderate/strong resistance against yellow rust were identified. During WGIN4, F3 bulked segregant analysis on the two most resistant Watkins genotypes (W733 and W786) will be carried out. In the 2018/19 Field Trial F_2 and F_3 families will be phenotyped to identify homozygous susceptible and resistant lines. Seedling virulence testing was performed at NIAB (Sarah Holdgate) and the results show that all 5 Watkins lines are susceptible at the seedling stage (**p41**).

Regarding recessively inherited *mlo* mediated resistance to powdery mildew, 4 of the double and 14 triple mutant lines were grown in the field to investigate whether there were trade-offs under field conditions with regards to foliar diseases, ear emergence and plant height. The parental line Cadenza anthesed up to 8 days before double or triple mutants. Cadenza also exhibited significantly more senescence, which could be related to earlier ear emergence. The Cadenza plots were between 5-10% taller than either the double or triple mutants. Although the powdery mildew disease pressure was low, flag leaves and second leaves were assessed and showed the expected reduction of disease for both mutant types. For brown rust, only a couple of triple mutants showed higher scores, but for yellow rust some double and triple mutants showed higher infection.

C. KHK – *mlo* in wheat has been engineered by gene editing by a Chinese group, but under the EU ruling these lines would not be allowed to be evaluated in the field. While the tilling approach took a lot long time, these mutant lines do not have any restrictions for field evaluation.

5. Update on Wheat Promotome Capture & *Tm* Introgression (MHK)

Details in presentation online (**pp53-74**).

5.1 Wheat Promotome Capture

Analysis of all promoters for traits 1, 2, 4, 5, 8 has been completed with regards to sequencing lengths obtained, Exon/Intron coverage, SNPs, INDELS, transposons, MITES, Repeat Elements and haplotypes observed for each promoter. Analysis of the other traits is ongoing.

Q. RB – regarding haplotypes, has this been done for a few genes only?

A. MHK – no, every promoter has been analysed. All data is available in the spreadsheet on Owncloud.

C. RB – this would be very valuable to look at.

Action MHK to send spreadsheet to RB and CL (nb: done Oct 11th)

The homoeologue specificity of MYbaits capture was re-emphasised (**pp54-55**) with two genes, AC3 (T4-57) and APG/OsPIL16 (T1-20) showing the coverage patterns in IGV. The effect of increased numbers of MYbaits per promoter was shown for 3 genes with only 1 bait, 8 baits and 26 MYbaits (the maximum number for this experimental design). Even with just one 120mer MYbait 895bp of sequence could be obtained. (**p56**).

The total lengths of sequence obtained was shown for promoters, 5'UTRs, Exons/Introns and total (**p58**) for 602 genes. In >98% of genes at least part of Exon 1 was consistently captured.

VRN1 (T7-37) was shown as an example regarding the limited number of haplotypes (**pp60-63**) with the A homoeologue having 8 haplotypes (only 4 with n≥3 varieties), the B homoeologue 6 haplotypes (only 3 with n≥3 varieties) and the D homoeologue 3 haplotypes (only 2 with n≥3 varieties).

Of the 506 genes received from the trait coordinators, 108 only had 1 or 2 homoeologues. For 78 of these, the missing homoeologues can now be identified via the Wheat eFP Browser (http://bar.utoronto.ca/~asher/efp_wheat/cgi-bin/efpWeb.cgi) resulting in 101 subsequent homoeologues. Of these, 83 have been captured in the WGIN promotome experiment, despite the fact that NO Mybaits were synthesised for these promoters. This is very similar to what Clare showed (see (2.) above). However, coverage (=number of aligned sequenced fragments) and sequence length are in most cases significantly reduced compared to the one (or two) homoeologue promoters for which the MYbaits were designed. RPA1a (T10-19) shows how this can be explained by mapping the MYbaits designed for the A homoeologue promoter to all three homoeologue sequences: for the A homoeologue all 14 MYbaits map without any mismatches (because the baits were designed for this promoter) whereas for the B and D homoeologue only 5 and 6 MYbaits map, respectively, with numerous mismatches and the coverage patterns observed in IGV can be explained by the actual position of the few mapped baits. (**pp66-68**). As the capture of these subsequent homoeologues can be explained thus, this seems to be an additional benefit of the experiment. Furthermore, it would be expected that any paralogues with at least some promoter sequence similarity have been captured and sequenced.

Q. RB – have you got all these varieties in a field trial?
A. MHK – No.
C. RB – if you did this you might be able to link any phenotype data to the promotome data (SNPs).

C. PS – referred to the planned June 2018 Promotome workshop, which was cancelled because too few people had managed to look at the data, and suggested to hold this workshop with the data MHK and CL now have, to show everyone what can be done.

Action: MHK – to set the workshop up again.

5.2 *Tm* Introgression

Of the 17 F_1C grains (crosses between the [*T durum* x *T monococcum*] F_1 hybrids with Paragon) obtained, only 11 grains germinated. Unfortunately, 5 of these seedlings did not grow and died. One

seedling did not grow initially and also appeared to die, but after several weeks a green shoot emerged and this has now grown into an anthesing plant. All of these F_1C plants are from F_1 hybrids with MDR031 only which exhibits Take-all resistance. Of these six plants, two have Kronos as a parent and the other four Hoh501. It is interesting to note that while the F_1 hybrid ears showed similarities to the *T. durum* parent, the F_1C ears are much more similar to Paragon (**p74**). Also, while the F_1 hybrid anthers had no pollen inside, the F_1C anthers do have pollen and these plants are partially self-fertile: 2 grains developed out of 13 florets which were not emasculated.

Q. PS – the numbers for pollinated stigmas in your table (**p71**) are very different for the three *T. monococcum* varieties. Does this mean that some of these crosses/ lines are more difficult to make?
A. MHK – No, the number of available ears/stigmas was limiting at the time. I would not expect any difference for various *Tm* accessions in generating F_1C grains. In the literature the expected rate of success is $\leq$0.5%.

Q. RB – do you know the genetic location of the traits of interest?
A. MHK – No.

C. KHK – when F_1 hybrids and F_1C plants come out of vernalisation they are put into an intermediate glasshouse to encourage additional tiller formation.
C. MHK – some plants now have 18 anthesing tillers.

Q. PS – do you know where Hoh501 comes from?
A. MHK – received from Richard Horsnell (NIAB), but know nothing else about this cultivar

Nb: VM contacted Richard: Hoh501 originates from Hohenheim University in Germany. 'It was actually Nick Gosman that sourced them. It is not a released variety but just a breeding line. We had a number of different Hoh lines but the 501 line seemed to work best when crossing to *Ae taushii's*. I think it was Friedrich Longin at Hohenheim who Nick obtained the seed from – he will know far more about their durum wheat breeding programme.'

6. WAK Gene Capture (KHK for KK)

Q. RB – is there any hypothesis yet about how these genes provide resistance against Septoria?
A. KHK – any mutations in the intracellular Kinase domain results in susceptibility. But there is also an extracellular domain which we hypothesise either directly or indirectly recognised the extracellularly growing Septoria.

The wheat genome contains over 600 of these WAK genes and Kostya has designed the MYbaits to cover all of these genes (Exons, Introns, UTRs) and their promoters. Kostya, with input from the breeders, has put together an initial list (98 cultivars) but has also received 50 lines from CIMMYT. As the total number of cultivars is limited to 96 lines (as in the Promotome Capture) Kostya would appreciate input from WGIN Management team members regarding which lines to keep and which to discard. The final decision will be made at the end of October.

7. The first RAG meeting

Q. PS – What is RAG?
A. KHK – RAG stands for Research Advisory Group. This is something completely new for the GINs.

Present at the first meeting were three people from Defra, the four GIN leaders and Harriet Trewin (BBSRC), David Cooper (ex Defra), Sean May (University of Nottingham), Bill Thomas (SCRI) and Dhan Bhandari (AHDB). The GIN leaders gave presentations about their intended projects for the next 5 years. Subsequently the RAG issued a document with 10 recommendations. Some of these were outlined by KHK. These RAG meetings will take place every 6 months for the duration of WGIN4, where the RAG will be checking on progress, outreach activities and opportunities to join the GINs together.

The first recommendation concerns the involvement of AHDB and how to interact with AHDB in different/ novel ways to insure the GINs are reaching out to new stakeholders. Also, interactions between the four GINs should involve sharing of procedures and experiences, eg regarding organisation of stakeholders' meetings. Potentially the organics sector industries could be interested in the zero N results.

C. MC – the first recommendation was mostly aimed at OREGIN. The RAG felt that the Stakeholder engagement aspect was a particular weakness of this particular GIN, but not WGIN or the other two.

The RAG also commented on the individual GIN websites and the need to keep these up to date. KHK mentioned the intention to have the WGIN website redesigned.

The second suggestion was to work with AHDB in bringing the crop genetics and crop management together. The third recommendation concerns Defra's press office, which needs to be more engaged with the GINs. Press releases need to be approved by the GIN leaders and images included in the press releases should come from actual GIN projects and not stock photographs. Recommendation 5 suggests that GIN leaders and /or members of GINs teams could be invited to the other GINs Management Meetings to find out more about the other GINs and link the four networks closer together.

C. PS – worth a try, but most likely won't lead to anything because everyone is already extremely busy.

Recommendation no.8 highlights the importance of Defra needing to be fully aware of all GIN publications, and Defra (Andy Cuthbertson) will maintain a database of GIN publications. Regarding WGIN all publications from WGIN3 are included in the final report, and all WGIN publications are listed on the WGIN website going back to 2004.

Q. MH – does this include publications that only acknowledge WGIN?
A. KHK – Yes, even if the main sponsor is different.

Q. MH – acknowledges WGIN quite often, but there should be a process in place to capture these publications as well.
A. KHK – any publication in press should be sent to Andy (Defra) and he will maintain this database.

Action: MC to correspond with RAG to find out what the exact process will be. Also, to determine whether this suggested publication database should be limited to publications that are largely funded by Defra or include those that use WGIN resources but are not funded by Defra, e.g. the drone work which mostly uses the Diversity trial site but is not funded by Defra.

Q. MH – how obliged are we to follow the recommendations by RAG?
A. MC – the idea is to at least consider the recommendations. If anyone decided not to follow them, they would need to come back to RAG with a rationale.

Q. MH – are these recommendations coming from the sponsor or the advisory group?
A. MC – they are coming from the RAG.

10. Stakeholders' Meeting

PS, KHK and MHK had a meeting to plan the 2018 stakeholders' event and decided to limit the number of presentations. Some of the DFW talks at the last SM were not really suitable for the stakeholders. Peter mentioned that the people we invite to give presentations actually know who they are talking to. This should not however involve talking down but balancing the content for the audience.

KHK – a reminder about this issue will be sent to all speakers.

The talks by WGIN scientists should both look back at the achievements of WGIN3 and forward to new WGIN4 projects.

C. PS – this time he would prefer somebody else, possibly John Knight (if he can do the introductory talk), to chair the discussion because he would be out of his comfort zone on chemistry.

Q. MHK – should we include summaries for each presentation in the programme as done for the first time last year?
A. PS/KHK – yes, that would be a good idea.

11. New publications, awarded grants and studentships using WGIN data and resources

12. Date of next WGIN Management meeting

A Doodle poll has been set up for February 12 – 14, 2019. Best date February 14th and to be held at Rothamsted Research.