WGIN 4 Management Meeting
8th May 2019 @ KWS (Thriplow)

Minutes

Attendees:
Peter Shewry (PS) (**chair**), Andrew Riche* (AR), Malcolm Hawkesford (MH), Gia Aradottir* (GA), Kim Hammond-Kosack*(KHK), Michael Hammond-Kosack* (MHK), Kostya Kanyuka* (KK) (RRes), Clare Lister* (CL), Simon Griffiths* (SG) (JIC), Charlotte Hayes (CH)(Elsoms), Sarah Holdgate (SH) (NIAB), David Feuerhelm (DF) (Syngenta), Simon Berry (SB) (Limagrain), Nick Bird (NB) (KWS), Sarah-Jane Osborne (SJ) (AHDB), Martin Cannell (MC) (Defra), Chris Burt (CB)(RAGT), John ??? (RAGT), Jacob Lage (JL) only for Gia's presentation (KWS)
[*=gave oral presentation]

Apologies:
Matt Kerton (DSV-UK), Ruth Bryant (RAGT), David Feuerhelm (Syngenta), Dhan Bhandari (AHDB)

A. Welcome – Peter Shewry

B. Presentations:

1. Gene Content around QTL loci

Details in presentation online (**pp1-13**).

QTLs have now been mapped to the physical and, importantly, fully annotated chromosome sequences (IWGSCrefseq1.0). This allows immediate identification of all genes within the QTL region. But depending on the size of the QTL region this could include several 100 genes.

For the **3A Height & Ear emergence** QTL (DTEM) in Avalon x Cadenza, the QTL region is ~60Mb and contains ~1000 annotated genes. Looking at genomic sequence close to the peak marker of the Ht QTL reveals a 2-3Mb region within the 60Mb with a grouping of 7 genes for cell wall synthesis which seem very likely candidates for a height QTL (**p7**).

The **6A Ear Emergence** QTL is least characterised. The QTL region contains ~1050 genes. This will still require fine mapping to narrow the QTL region down. A number of putative gene candidates which might be involved in flowering have been identified (**p10**).

The **2D Height** QTL (= Rht8) has been fine-mapped to 3Mb containing ~30 genes but also a lot of transposons. Two possible candidates at either end of this region are **scarecrow protein 3** and **Giberellin GA5**.

Q. PS –Which tissues were used?
A. SG – internode and inflorescence. Height is determined by the length of internodes.

Q. KK – how did you reduce 1000 genes down to 6?

A. CL – this was done by manual selection based on the annotation gene function and then looking through for plausible candidates.

C. ?? – there is also a GA (Gibberellin) gene in the middle of the cell wall genes which could also be a likely candidate.

C. SG – when introgressing the 3A QTL, the yield dissolves away.

C. MHK – at MonoGram2019 the importance of de novo assembly of sequenced wheat cultivars was stressed. It could be that the QTL regions are shorter if mapped directly to Cadenza and Avalon.

Q. SB – have you looked at Rht8 mutations?
A. CL – not yet, but would be definitely worthwhile

C. SG – would like to do long-range sequencing of QTL regions

C. KK – we could use VIGS to validate any of the candidate genes.
A. SG – very happy to accept this collaboration offer.

2. Genetic dissection of drought tolerance (SG)

Details in presentation online (**pp14-22**).

Because of the spring drought in 2019, there is already an effect visible in the field. The grain number is determined during spring with florets developing between April and May. In 2018, when the drought occurred during grain filling, the effect on yield was reduced because in April there were over 40mm of rain. Garcia has a drought QTL on chromosome 2B which is not ppd1 related.

Any varieties from WGIN can be nominated for the DFW Toolkit, in fact any wheat project can do this.

Canopy Temperature (T) correlates well with yield with strong QTLs on chromosomes 4D and 5A. Reduced canopy temp alleles correspond to large grain size (**p20**). High yielding plots are the cooler ones on hot days. The thermal camera (on drone flights) can pick up the thermal alleles. The next drone flight is planned for late May (AR). It would be very useful to categorise the TGW QTLs with thermal data. For the 2019/2020 season the drought trial has been designed with replicated yield plots ($6m^2$) with and without irrigation to determine which parents show reduced yield penalty under drought (**p22**).

C. MH – these measurements are taken on several flights in one day. As the weather conditions are different from year to year, this would make correlations between years very difficult.

C. MH – extracting this information from the raw data is not straightforward and thus time-consuming. Doing this would require additional personnel.

Q. PS – does grain size effect yield
A. SG – generally grain size QTLs are not coming though as higher yield QTLs.

Q. KHK – what was the amount of rain this April?
A. CL/ SG – not sure about exact amounts, but the differences between irrigated and non-irrigated plots are clearly visible.

C. MH – there could also be an influence of winter rainfall, ie this could lessen the effect of spring drought.

WGIN4 includes a variety panel for drought and lodging (DALP) (**p21**). An oat cultivar is included because oat has exceptionally good anchorage (very long/deep roots).

C. CL – if anyone has an idea about how to measure lodging effectively, please let us know. Currently we are just measuring anchorage strength with a pull meter.

3. WGIN Diversity trial 2018/2019 focus - N use efficiency (AR)

Details in presentation online (**pp23-36**).

This year's Diversity Trial has changed in that it includes duplicate plots **with and without fungicide treatments**, to determine the effect of Nitrogen on disease occurrence. Because of this, plot sizes were halved to 1.5 x $9m^2$ (from 3 x $9m^2$ in all previous years). Drone flights are taking place in weekly intervals.

Grain Protein Deviation (**GPD**) is a genetic trait which refers to the fact that while most wheat cultivars show a negative correlation between grain protein content and yield, a few varieties show a positive correlation (hence 'deviation' from the norm). Ellen Mosleth et al devised a statistical method to remove year effects and correct for fertiliser N as shown (**p28**) called corrected GPD (Plant Biotechnology Journal (2015) 13, pp. 625–635 doi: 10.1111/pbi.12285). Hereward has a high corrected GPD already at N0.

Q. SH – are you looking for disease in general or specific diseases?
A. AR/ MH – specific diseases on specific days on the ground and using the drone for general disease symptoms.

Q. SG – what is your hypothesis regarding disease occurrence and N?
A. all – the expectation is to have more severe disease symptoms at higher N regimes.
C. MHK – Gustavo Slafer showed at MonoGram that the drought penalty increases also with higher N input.

C. MH – 10 of the cultivars have been consistently used in the Diversity trials, while the others have changed over the years because the rationale has changed over 16 years.

Q. SG – is there any interest in the genetics of GPD in the UK?
A. SB/ CB – not so much here, mainly in France and Germany.

4. Resistance to BYDV transmitted by Aphids (GA)

Details in presentation online (**pp37-42**).

BYDV infection was assessed for the 20 WGIN Diversity cultivars in the glasshouse (**p39-40**) on two dates (February13th and May 6th), but no obvious differences could be detected. The next batch of cultivars to be tested includes 18 Watkins lines (**p41**) selected from aphid phenotyping. But Gia would happily include other cultivars if nominated by breeders. It is also planned to visit farmers for BYDV sampling (involving AHDB).

Q. SB – what is the scoring scale?
A. GA – Zero to 9

Q. KK – what is scored?
A. GA – leaf yellowing (see image on **p38**).

Q. SH – how does the virus get into the plants?
A. GA – via aphid feeding (72h) before vernalisation.

C. SH – you could ask farmers to send samples to you instead of visiting farms yourself.

Q. KK – is there any known resistance to BYDV in cultivated germplasm?
A. GA – No – that's why the next batch of cultivars includes mainly Watkins lines. Also had asked for introgressed cultivars from Nottingham, but had no response.
C. SG – most of these (more than 120 lines) are available via the GRU.
C. NB – but not the original lines.

C. SH – Dr Laura Flint (Molecular Plant Virologist at Fera Science Limited**)**
did her PhD on BYDV and might be worth contacting.

5. Update on *T. monococcum* introgression (MHK)

Details in presentation online (**pp43-49**).

Tm Introgression is now at stage 4 (BC_2) for MDR031 (Take-All) with 28 plants in the glasshouse, of which some Kronos derived ones have already been pollinated and so far, 9 BC_2 grains are developing.

For MDR049 (aphids) and MDR308 (Septoria) introgression is at stage 3 (BC_1), with 1 and 5 F_1C plants, respectively, in the glasshouse.

6. Wheat genome exploitation through genomics analyses (KK)

Details in presentation online (**pp50-57**).

6.1 Genome-wide sequence analysis of WAKs using exome and promotome capture

The 96 cultivars used for this experiment include 20 exotic wheats carrying known Septoria resistance genes, 14 Watkins lines showing high level of resistance to multiple foliar diseases, 60 wheat cultivars mostly of UK/ European origin with known field reaction to Septoria and 2 Triticum monococcum genotypes with contrasting response to Septoria (**p52**) [nb: MDR002 (sensitive) and MDR308 (DV92) (resistant)].

The sequencing data have already been received from Arbor Biosciences. As of May 7th, Illumina 150 nt reads have been trimmed for adaptor contamination and for quality at the 3' end. Reads shortened to less than 40 nt were discarded. This produced more discards on the reverse reads (this is typical). The loss rate of reverse reads was <10% with fewer than 2% of read pairs discarded. Reads were retested for quality with FastQC and all issues were resolved. Paired reads were mapped with BWA (v0.7.17) to the

IWGSC release 43 reference (cv. Chinese Spring genome). The data is ready for sequence depth analysis and presence/absence determination, and SNP calling which will be done very shortly (June 2019).

6.2 Triticum monococcum chromosome 7Am sequencing and long-range assembly (towards isolation of TmStb1)

Regarding the cloning of *TmStb1* (from MDR308), this disease resistance gene resides on Tm Chr $7A^m$, but the locus is not fully genetically defined because the interval is several cMorgans long and nearby the centromere. However, faster cloning of wheat genes is now possible via long-range chromosome assembly. Currently, the entire Tm Chromosome $7A^m$ is being isolated via flow sorting by the Institute of Experimental Botany CAS (Olomouc, Czech Republic) (**p55**). Isolation of sufficiently pure Chr $7A^m$ has been promised by August 2019. The sequencing and long-range assembly of Chr $7A^m$ will then be carried out by Dovetail Genomics (Santa Cruz, CA, USA) using the Chicago methodology which involves *in vitro* reconstituted chromatin to achieve highly accurate chromosome assembly (http://www.genome.org/cgi/doi/10.1101/gr.193474.115).

Q. CB – has the resistant *Tm* cultivar been included in the Tm Introgression experiment?
A. MHK – yes, it has, this is MDR308.

Nb: as of June 1st 2019 there are now 101 BC_1 grains ripening for MDR308 (MHK)

7. Feedback from the Defra joint GINs 1st year evaluation meeting - March 2019 (MC)

8. Stakeholders' Meeting 2019

Action (MHK): Doodle poll to set up for November 13-15th **and** check with RoCRE conferencing about availability of Fowden Hall.
n.b.: done Thursday May 9th

The topic for the Panel Discussion will be '**Loss of Insecticides**'.

SH would like to see some gender balance on the panel. With this in mind the following where suggested:

1) Lyn Field (RRes), also to give introductory talk
2) Gia Aridottir (RRes) –agreed to take part, but has subsequently dropped out
3) Ruth Bryant (RAGT) – volunteered in here absence
4) Farmer – tbc

Regarding speakers the following were suggested:

1) Raul Bhosale – roots (Nottingham Uni)
2) Peter Shewry – changes in UK wheat (RRes, already agreed)
3) Toby Bruce (University of Keele)
4) Laura Dixon (ex JIC, now at University of Leeds)
5) Phillippa Borrill (University of Birmingham)
6) AHDB Strategic Farmer – tbc
7) Wheat breeder – tbc

8) Nick Fladgley – NIAB wheat pedigrees
9) Tom Bennett (Leeds University)

Regarding content of presentation the need to keep it at an understandable level (similar to last year) to be stressed at the speaker invite stage.

9. New publications, awarded grants and new studentships using WGIN data

- Jess Spong, Take-all, Nottingham DTP (KHK)
- AHDB studentship on genetic and environmental effects of protein amount and quality in wheat. The project will use the Malacca x Hereward population for QTL analysis of GPD and near-isogenic pairs developed from this population to identify the mechanisms and underlying genes of quality QTLs. The student, Rohan Richard, has experience working at INRA Clermont-Ferrand.
- MH & SG – DFW paper acknowledging WGIN
- MH – other publications in progress

10. Review of year 2 milestones Gantt chart

Milestone 25 is not possible to be completed by September 2019.

Action: This may / may not necessitates a contract change – Martin, Simon, Claire and Kim to discuss.

10. date for next WGIN Management Meeting

This meeting will take place on **Tuesday, October 8th 2019 at JIC (venue - Chris Lamb Suite)** .

Nb: venue agreed at this MM, date determined via subsequent Doodle poll.

11. AOB