WGIN 3 Management Meeting 2
17th July 2015 @ Rothamsted Research, Harpenden

This was the second Management Meeting of the newly **DEFRA** funded **WGIN3**.

Draft Minutes

Attendees:-
Peter Shewry (PS) (chair), Andrew Riche*(1) (AR), Lesley Smart* (LS), Kostya Kanyuka (KK), Vanessa McMillan* (VM), Kim Hammond-Kosack* (KHK), Michael Hammond-Kosack (MHK) (RRes), Simon Griffiths (SG), Clare Lister* (CL), Alba Farre Martinez* (AFM) (JIC), Jacob Lage (JL) (KWS), Alison Bentley (2) (AB) (NIAB), Matt Kerton (MK) (DSV), Dhan Bhandari (DB) (HGCA), Ellie Marshall (EM) (HGCA), Simon Berry (SB) (Limagrain), Simon Penson (SP) (Campden BRI), Giulia Cuccato (GC) (Defra)

[*=giving presentations, (1) dep for Malcolm Hawkesford, (2) dep for Sarah Holdgate]

Apologies: David Feuerhelm (Syngenta), Ruth Bryant (RAGT), Sarah Holdgate (NIAB), Jayne Brookman (KTN), Malcolm Hawkesford (RRes)

A. Welcome – Peter Shewry

B. Presentations:

1. Brief Introduction to the WGIN 3 project (KHK)

The key emphasis of WGIN 3 is **yield** stability and the working title of WGIN3 is: ***'Defra Wheat Genetic Improvement Network - Improving the resilience of the wheat crop through genetics and targeted traits analysis'***

There are **four work packages** (details on presentation slide), **WP1** – Management, Networking & Communication, **WP2 & 4** – Genetic and QTL analyses of targeted traits and **WP3** – Tools and Resources. WGIN 3 consists of **2 project partners**, The John Innes Centre and Rothamsted Research and **2 new subcontractors**, Bristol Genomics Facility (University of Bristol) and MYcroarray (Michigan, USA).

Within these work packages there are **five** topics new to WGIN:

- AxC NIL Tiling Population (WP3.2)
- Yellow Rust Resistance (WP2.2)
- Gene specific marker development (WP2.4)
- NGS genome/exome capture (WP1.3 & WP4.2)
- Industry led forum (WP1.5)

WGIN 3 contains **21 milestones**, with individual target dates between March 2015 and December 2016 (details on presentation slide).

Q. DB – were any major questions raised at the April Stakeholders' Meeting?

A. KHK & PS – no, quite a small meeting, main discussion on approaches to be used for the exome capture.

2. Giulia Cuccato (Defra)

Giulia gave a brief update on the political/governmental perception of WGIN. Defra is picking up more on WGIN and GINs generally and is viewing them favourably. It would be very important to keep WGIN in the spotlight (with a view to further funding).

GC suggested a joint GIN meeting (WGIN, ORIGIN, VeGIN, PCGIN (pulses)) to showcase the importance of GIN research for economic growth, preferably at one of the two research centres (JIC or RRes) so that politicians/ministers could see directly where activities are done. Generally this suggestion received a positive response. It was discussed whether this could be combined with the next Stakeholders' Meeting but decided to keep them separate, with at least a month between the two meetings.

Action Item: KHK to initiate contact with

- Claire Domoney (PCGIN)
- Ian Bancroft (OREGIN)
- Graham Teakle (VeGIN)

3. WGIN NIL Analysis - Understanding genotype x environment interactions in Avalon x Cadenza (AFM)

QTLs controlling heading date (HD), plant height (PH) and grain yield (GRYLD) were identified in Avalon x Cadenza DH population. Eleven target regions were selected based on these results. A total of 553 BC2 NILs were generated (250 and 303 NILs with Avalon (winter wheat) and Cadenza (spring wheat) background, respectively).

Field trials were conducted in two growing seasons (2012/2013 and 2013/2014) which differed in terms of temperature and precipitation during the growing cycle. Principal component analysis (PCA) was use as a graphical tool to study the similarities between environments. In 2013 all NILs were mixed whereas in 2014 the NILs were divided into two groups depending on the background (Avalon and Cadenza). NILs carrying the Cadenza background were taller, flowered early and had increased GRYLD and yield components in 2014. Part of these differences can be explained by Vrn-A1 (on 5A) and Rht-D1 (on 4D).

GRYLD was driven by GRpsqm as all the chromosomes that showed significant differences between alleles for GRYLD were also significant for GRpsqm (1B, 1D, 3A and 7B). The same effect was observed for GRYLD and GRpsqm. However, the increasing allele on 1B and 3A was Cadenza whereas on 1D and 7B the increasing allele was Avalon.

In summary, significant differences were observed between the Avalon and Cadenza alleles. This means that Avalon x Cadenza QTL for height, heading date, grain yield, and grain size/shape have been converted to discrete Mendelian factors which are now amenable to high resolution genetic mapping.

4. A chromosome segment substitution library (CSSL) for Avalon x Cadenza (CL)

The sub-projects carried out by the group of Simon Griffiths consist of

1) Dissecting UK drought tolerance in Paragon x Garcia
2) Quantifying agronomic impact of WGIN target genes using the Paragon NIL library
3) Informing multiple marker assisted selection for yield stability using Paragon library
4) A chromosome segment substitution library (CSSL) for Avalon x Cadenza
5) Understanding genotype x environment interaction in Avalon x Cadenza –As described in Alba Farre's presentation.
6) Foundations for a new generation segregating populations for studying yield stability in the UK
7) Applying WGIN data to breeding by design for UK yield stability
8) Curation and distribution of WGIN germplasm

Clare Lister's presentation focussed on the generation of the CSSL. WGIN successfully promoted the A x C DH population as the UK reference population. The A x C population is the most densely mapped in the world. Much phenotypic data is also available. The NILs derived from these have validated QTLs. The BC3 NILs carry selected genetic foreground in the QTL regions (height, heading, and yield). In addition each line carries ~12.5% random chromosomal regions.

A total of 94 lines consisting of 47 Avalon+b allele and 47 Cadenza+a allele lines representing all the QTLs (EM, Ht, YLD) were selected for CSSL. Requirements for CSSL are a full AxC Map (18 942 markers), a Frame AxC Map (1 286 markers) (both from Bristol) and Genotyping of 94 NIL lines (on 35K Axiom array).

18 maps (from 35k and 820k AxC array data, plus additional markers from Frame Map not in 35K array) are already nearing completion and will be ready in mid-August. They will be made available on the WGIN website. Eventually, all 94 maps (from 35k and 820k AxC array data only) will be available. Subsequently, lines will be backcrossed twice to the recurrent parent, KASP markers will be used to select for the new target segment, lines will be self fertilised and homozygous CSSLs selected and all generated lines will be available for use.

Q. ? – has a tiling path been determined?

A. CL – all 94 lines were chosen because they are already at BC3 stage

C. AB – NIAB is about to release new maps based on the NIAB MAGIC population and this additional information could/should be included.

Q. KHK – what is covered by the 18 lines and which portions are missing?
A. CL – still need to do this analysis

5. Nitrogen Use Efficiency & Diversity (AR)

The trial will be continued with 25 varieties in the autumn of 2015 (details in presentation on WGIN website) but another five lines (one from each breeder) will be included for a total of 30 lines. The new varieties are Evolution (Limagrain), Lili (KWS), Reflection (Syngenta), RGT

Illustrious (RAGT) and Hylux (Saaten Union). These were chosen after assessing characteristics at Cereals 2015.

The aerial imaging trial is continuing, focussing on plant height measurements. The aim is to generate **orthomosaic images** [an aerial photograph geometrically corrected ("orthorectified") such that the scale is uniform: the photo has the same lack of distortion as a map. Unlike an uncorrected aerial photograph, an orthophotograph can be used to measure true distances, because it is an accurate representation of the Earth's surface, having been adjusted for topographic relief, lens distortion, and camera tilt]. Requirements for the generation of such images are typically 500 photographs per experiment with 80% overlap and 12 GCPs per experiment [ground control points – 2 black and two white paving slabs per GCP]. Assembly is processor intensive and can take over 24 hours.

Currently the resolution is 1cm per pixel at a flight height of 40ft. The resolution could be increased by lowering the flight height. At present there is a very good correlation between auto and manual height measurements **provided** the canopy is complete with plant heights of >60cm. However for sparse plots and low plant heights auto and manual measurements can differ by a factor of 2.

Data collection for 2015 include Spectral reflectance (weekly), date of anthesis, senescence, canopy height, N & mineral uptake during GFP, aerial images and final harvest grain and straw yield.

Q. VM – do any varieties have long awns and does this interfere with measurements?
A. AR – 2 varieties do, but not checked yet

Q. DB – could aerial imaging be used to assess head blight?
A. AR – potentially yes, but resolution would have to be increased significantly

Q. DB – is it worth reassessing varieties for useful data regarding head blight?
A. KHK – all varieties/plots are fully fungicide treated, so these data sets are not useful for assessing diseases.

6. Screening germplasm for resilience to aphids (LS)

The research focusses now on *Triticum monococcum* lines because these provided the most promising leads for partial resistance to cereal aphids from previous work. Crosses of two fully (MDR045 & MDR657) and one partially (MDR049) aphid resistant Tm lines to a fully susceptible one (MDR037) were made by Mike Hammond-Kosack: MDR037 x MDR045, MDR037 x MDR657 and MDR037 x MDR049. F_1 generations of these crosses have now been tested in the phenotyping screen along with parental lines against both aphid species. Analysis of *Rhopalosiphum padi* mean nymph weight (mg) after 6 days on *T. monococcum* lines and crosses unfortunately did not show any significant differences, but more importantly showed nymph production on MDR045 and MDR657 which previously had none at all. This could be due to the plants being older during this phenotyping screen. Interestingly, *Sitobion avenae* nymph weight screens showed some differences, notably with MDR049 still showing reduced nymph weight gain (partial resistance) in comparison to the control. So there appears to be a separation for both aphid species in the Tm crosses.

F_2 generations and backcrosses to MDR037 have just been harvested and will be tested in phenotyping screens and taken to further generations.

Metabolomic analyses (funded by IDG) of whole leaf tissue (both undamaged controls and aphid damaged) were carried out for Solstice (fully susceptible) and MDR037, MDR049 and MDR657. PCA analysis revealed that both susceptible varieties (Solstice, MDR037) and partially and fully resistant varieties (MDR049, MDR657) group together, both before and after 24h aphid infestation. Furthermore, there are several elevated components in the (partially) resistant lines, the most elevated being (in decreasing order) choline, glycerol, octopamine, threonine, glutamine, asparagine, guanosine, succinate and trehalose. Further work is planned to investigate effects of some of these chemicals against aphids in feeding bioassays.

C. KHK – age effects on resistance are a well documented phenomenon for various plant pathogen interactions.

7. Resistance to Yellow Rust (VM)

7.1 Objective 3: Examine the resistance of *Triticum monococcum* to yellow rust

Wheat yellow rust (*Puccinia striiformis* f.sp. *tritici*) is an obligate biotrophic pathogen which causes yield losses of up to 50%. Since the start of WGIN a *Triticum monococcum* collection (323 lines) has been established at RRes. No obvious yellow rust infection has been observed. 263 lines (not enough seed available for the remainder) were sown in the autumn of 2014 and 216 lines were scored for yellow rust under field trial conditions: Diverse *T. monococcum* accessions were all highly resistant to yellow rust. Low levels of yellow rust (1% severity) were detected at tillering and stem elongation/flag leaf emergence for many accessions. 99% of accessions showed resistant phenotype at flowering (some chlorosis/necrosis, no yellow rust sporulation). Only two accessions showed yellow rust sporulation on flag leaf at flowering [MDR634: 10% - probably not *T. monococcum*, mistake in seed store or original accession received; whilst MDR288 which appears to be a *T. monococcum* : 2% - also showed stem purpling and powdery mildew infection].

C. JL Suggest not to repeat the trial in year 2 because this data suggests *T. monococcum* is a non-host species to the wheat infecting species *Puccinia striiformis* f.sp. *tritici*

7.2 Objective 4: Characterise hexaploid wheat germplasm previously shown to exhibit a high level of resistance to multiple foliar diseases

The Watkins 2008 field trial identified 10 Watkins accessions (out of 740) with a high degree of resistance to all 4 foliar pathogens. However, as this was a 3rd wheat trial and also a high take-all disease year with root infection early in the season, it is possible that the foliar disease resistance was an induced plant response. Ten Watkins lines and controls were sown in both 1st wheat (no take-all) and 3rd wheat (high take-all) field trials in autumn 2014. No fungicides were applied to allow natural disease to develop and all lines were scored for foliar diseases and take-all. 5/10 Watkins lines were very susceptible to yellow rust (with lines 18 & 495 showing low disease severity in May) while 4/10 showed some resistance (lines 203, 231, 610 and 786) and one line, 733, showing no disease symptoms. All resistant lines (apart from line 786) as well as lines 18 and

495 have been crossed in the field to the highly susceptible variety Fielder which showed close to 100% spore coverage of the flag leaf and 250 seeds were harvested (MHK).

Watkins line 786 will be crossed with cv. Fielder in the glasshouse or field in 2016 (not included in 2015 field crossing due to high disease in May) and the mapping populations will be progressed to F_2 and then screened for yellow rust resistance.

C. JL&AB – *Tm* apparently is a yellow rust non-host, and therefore it would not be useful to continue this work, but focus on Watkins lines instead.

Q. JL – has take-all been seen in this latest trial?
A. VM – this has not yet been assessed.

8. Summary of exome capture discussions @ WGIN Stakeholder meeting 16th April 2015 (KHK)

As exome capture has already been used for wheat genes, it was decided to focus instead on regulatory elements, i.e. promoters (up to 1kb) and possibly some known regulatory introns with the aim of capturing the A, B and D genome sequences individually.

A designated group of WGIN scientists will interact with the company MYcroArray to decide on the best way to represent wheat gene promoters on the 20,000 bit array. It had been decided on April 16 to focus on the following 8 trait categories with 50 promoter/gene sequences to be nominated for each, ideally by one representative for each trait. The names listed below next to each trait category were suggested/ agreed upon at this MM:

1)	Yield resilience	Cristobal Uauy, need a 2nd
2)	Grain quality	Peter Shewry, Kay Trafford (NIAB), Rowan Mitchell (RRes)
3)	Biotic stress – fungi and insects	KHK, Brandt Wulff (JIC), Matthew Moscow (SL)
4)	Abiotic stress – drought, high temp	John Foulkes, need a 2nd
5)	Nutrient use efficiency	Malcolm Hawkesford and Nottingham
6)	Canopy development(whole plant architecture)	SG, AB, Andy Phillips
7)	Flower biology	Zoe Wilson, need a 2nd
8)	Root architecture	Malcolm Bennett, need a 2nd

Action item: KHK to contact potential trait representatives

Q. SP – would this be phenotyping by panel?
A. PS – the lines and the genes would be selected by each representative based on prior knowledge / peer reviewed publications

C. Stakeholder Meeting

The second WGIN3 Stakeholder Meeting will take place on **Friday November 20th @ Rothamsted**.

The only and agreed upon Panel Discussion Topic was **Nutrient Uptake** which should be chaired by Malcolm Hawkesford.
It was also agreed that WGIN should aim to have any newly funded external wheat projects represented at the stakeholder meeting, possibly with each PI to give a 10 -15 min talk.

Action Item: MHK to contact the following for further details:

> UK Agri Innovations Centres – AgriMetrics (John Crawford, RRes), Crop Health and Protection (CHAP) (Budge, Fera or Lin Field, RRes)
> link projects – JIC, ADAS, RRes
> AHDB new strategy emerging (-> Dhan Bhandari)
> BBSRC responsive mode (-> website)
> Universities of Bristol, Durham (->Ari Sadanandom), Nottingham
> SARIC (Sustainable Agriculture Research and Innovation Club) (-> Stephen Norton)
> NIAB – Magic (-> James Cockram)

Action Item: contact and invite the media, farming press to stakeholder meeting

Action Item: MHK to send Stakeholder group email list to all Management team to update.

D. Overseas joint workshop (PS)

Peter explained that the proposed trip to Russia and the Vavilov Institute was unlikely (no direct contacts anymore). Instead, Japan and Turkey were proposed with Turkey currently being the front runner. A BBSRC travel application will be submitted by Peter this autumn (Deadline date expected to be November)

Version 2 14th August MHK + KHK+PS