



**WGIN Management Meeting
22nd November 2005
Rothamsted Research, Harpenden, Herts**

MINUTES

Attending

Management Team

John Snape, Andy Phillips, Kim Hammond-Kosack, Bruno Viegas, Rosemary Bayles, Neil Paveley, Peter Jack, Chris Chapman, Graham Jellis, Tina Barsby, Stephen J Smith, Chris Tapsell, Philip Howell, Andy Greenland, Chris Chapman, Nick Balaam, Peter Shewry

Observers

Hai-Chun Jing, Simon Orford, Leodie Alibert

Report on year progress and research goals

Andy Phillips (RRes)

Objective 9 TILLING in hexaploid wheat

Powerpoint Presentation: 001 Andy Phillips (RRes) TILLING

To date, three EMS populations of the hexaploid spring wheat Cadenza, in total 5,000 M₂ lines, have been generated. The TILLING platform has been established at Rothamsted Research after solving several technical challenges including designing (homoeologous) gene specific primers and optimisation of PCR conditions using IRD-labelled primers and CELI concentrations. As a proof of concept, GA20ox genes have been TILLED in the EMS populations and variant alleles found. From mid 2006, the platform will be developed into an RRes-based Wheat TILLING Service, covering hexaploid wheat. At a later date this TILLING service will extend to tetraploid (*T. durum* cv. Cham1) and diploid (*Triticum monococcum* line DV 92) wheats. Eco-TILLING will also be performed for diploid wheat and recommended list varieties. The estimated FEC cost for TILLING in EMS-mutagenised populations of hexaploid wheat will be in the range of £2,500 per homoeologue per 1,000 lines screened.

Questions

Q1. *In one of your examples, a heterozygote was used for mismatch detection, is it desirable to keep the EMS populations as early generations?*

We would normally use pools of DNA from multiple individuals and hence homozygous mutations should also be detected. However, it would be beneficial to keep the early generations of EMS populations, especially when you expect to have lethal mutations.

Q2. *Is it possible for the Wheat TILLING Service to provide confidential service for companies?*

Yes, if the request is from outside the WGIN framework. In the long term, the TILLING service will be developed into a platform which has a high enough workload to keep at least one permanent member of staff. This service would be open to both commercial and public sectors, but it would be important to ensure that private sector needs did not restrict availability of the service to public sector organisations

Q3. *After proof of concept using the mentioned candidate genes, is there any plan to include more target genes that control important yield and quality traits?*

Yes, this point has been discussed in-house. More effort has been made to define new targets for TILLING. In WGIN Year 3, new targets have to be agreed and discussed in consultation with the Management Committee.

Kim Hammond-Kosack (RRes)

Objective 6 *Triticum monococcum*

Powerpoint Presentation: 002 Kim Hammond-Kosack (RRes) DIPLOID WHEAT

The diploid wheat *Triticum monococcum* represents an AA genome related to that of hexaploid bread wheat. The aim of this research activity is to develop *T. monococcum* into a reference species for gene functional studies, for discovery of novel variant alleles and for establishing specific genotype:phenotype associations. In addition to the 124 accessions which have been collected mainly from Vavilov Institute, St Petersburg, Russia and the USDA Small Grains Centre, another 139 lines were acquired from IPK, Germany. Furthermore, 72 CIMMYT lines have been ordered. To establish molecular tools, 128 SSR markers from the A genome of bread wheat were selected and 96 tested in *T. monococcum*. The results showed that a large portion of the SSR markers are transferable. The genetic diversity of the *T. monococcum* collection and the 30 lines selected as a core collection (based on pathogen resistance phenotyping results) has been assayed using 22 SSR markers and an overall of 25% genetic diversity found. Furthermore, five different mapping population and an EMS population of 2000 M₁ plants have been generated. A selected *Triticum monococcum* line will be sent to China for physical mutagenesis using a unique ionising irradiation method.

Questions /Comments

C1. Your SSR data suggest that genetically the *Triticum monococcum* accessions of the Vavilov collection are rather uniform. This could be due to the artificial selection done in the Vavilov Institute over the years and may not necessarily reflect the original polymorphic levels.

Q1. What type of ionising irradiation methods are used in the Chinese institute?

There is very little information available in English. Additional efforts will be made by KHK and H-C Jing to obtain information on the exact procedure and the mechanisms underlying this physical mutagenesis method.

Q2. What kind of traits did they select for after using this physical ionising method? Are any mutagenised hexaploid wheat populations available?

The mutagenesis was done on hexaploid Chinese lines. The breeders selected for several traits including increased quality for making steamed breads. These selections were successful and commercial cultivars were released and did well in many regions of China. KHK will find out what other traits were selected for.

Q3. Would this method create a higher mutation rate than EMS? Can we send both *Triticum monococcum* and a UK bread wheat variety (eg Cadenza) to China for a pioneer experiment? How efficient is this method?

This is not clear. However, it is possible and sensible to have UK-based varieties treated and tested to judge the efficiency.

The management team decided that it was appropriate to send both *Triticum monococcum* lines and Cadenza hexaploid wheat for the physical ionising treatment.

John Snape (JIC)

Objective 2

Powerpoint Presentation: 003 John Snape (JIC) WATKINS and AxC

The Watkins Collection consists of 900 lines purified by individually bagging four plants/line. Furthermore, 4 plants from each line, in total 400 lines, were genotyped with 10 SSR markers. Overall, 10% heterogeneity was found.

An EMS population of Paragon has been generated. From the 3,500 M2 lines, two plants per line were grown and 6,500 lines remain. The M6 generation will be available from early 2006. Phenotyping on height, ear, and time to germination has been performed. As a proof of concept, the *Rht* genes have been examined to estimate the mutation rate using SSCP screening. In one case, three mutations were found in the *Rht-B1* gene.

Differences in straw wall thickness (at 10 cm below the ear) have been observed in a range of wheat varieties. Taking advantage of differences between the Avalon (thin) x Cadenza (solid) parents the DH population was

scored for segregation. A major QTL has been mapped to chromosome 3B linked to SSR marker *Xgwm547*.

Questions/Comments

Q1. *Has any phenotyping work been done on the AxC mapping population?*

Drilled 5 m plot field trials were grown over the 2004/05 growing season and the data on a range of characters, including yield components collected and are currently being analysed. Kim's group has been phenotyping the resistance to *Mycosphaerella graminicola*. Peter Shewry's group has taken 10 lines with variation in the straw wall thickness to monitor nitrogen dynamics in straw in relation to nutrient storage and relocation prior to anthesis. The current hypothesis is that straw thickening may act as transient nitrogen storage. In addition, the population has been scored for a number of canopy architecture traits by Darren Lovell.

It was agreed that a list of traits scored using the Avalon x Cadenza parents and DH population should be drawn up and maintained on the WGIN website.

Q2. *Is it possible to share the information on the straw nitrogen dynamics?*

Yes, currently the experiment is running. The next management meeting should be a proper time to report the results.

Q3. *Is the thickness of the straw very uniform from base to top?*

The current assessment was done on the part 10 cms below the main inflorescence. It tends to get thinner towards the bottom of the straw. However, more detailed examination is required.

Q4. *Is straw wall thickness correlated to lodging resistance?*

The correlation is weak.

Objective 3 Generation of a reference double haploid mapping population

Avalon x Cadenza was selected in 2004 as a core mapping population in the WGIN project. In total, four sets of samples have been subject to SSR mapping. Furthermore, DarT markers (see Management meeting minutes May 2005 for a full description of this technology) have been developed for the population. Further information on DarT markers can be found at <http://www.diversityarrays.com>). It was found that most of the DarT markers co-segregate with SSR markers. In total, 100 SSR and 256 Dart markers have been placed on the entire AxC mapping population.

Questions/Comments

Q1. *Why are the DarT markers tightly clustered into several genome regions?*

This may have something to do with the way the markers were selected by the producer, although the company claims that the markers are randomised across the genome. The main reasons to choose DarT markers are that they have good coverage and are relatively cheap.

Q2. *Does the company provide sequence information on the markers?*

They may supply it but on a case by case basis, rather than providing the whole set of information.

Q3. *How do you send your samples over to Australia?*

The company has specific forms and documents to complete but the process is relatively straightforward.

Q4. *What do you know about the variation in the important traits in the A x C population?*

There is a range of disease scoring data. Other traits include dwarf phenotype and preharvest sprouting. See also the previous section.

Objective 4

John Snape also reported work on the development of COS markers, which are intron-based and are aimed to distinguish homoeologous genes in wheat. A range of bioinformatics tools including mining the wheat Bin-mapped ESTs and ITMI EST contigs and blasting the rice genome have been used to define possible intron-exon boundaries and to develop primers specific for homoeologous genes. The primers are also effective in a variety of species including wheat, rice, barley and *Brachypodium*.

Questions/Comments

Q1. *What is the level of polymorphism in COS markers in wheat?*

Similar to SSR markers.

Q2. *How many COS markers have been developed?*

Currently about 30. We are hoping to develop more in collaboration with IDNA Genetics (Peter Isaac)

Neil Paveley (ADAS)/Rosemary Bayles (NIAB)
Objective 5 TRAITS - Sub-contract Second Wheat Syndrome – Take-all Year 1

Powerpoint Presentation: 004 Neil Paveley (ADAS) 2nd wheat syndrome

Second wheat syndrome was identified during the Traits meeting held in June 2004 as having high priority for many of the breeders. Field trials on second wheat syndrome were carried out by ADAS and NIAB in 2004/5 with advice and input from Bill Hollins at RAGT and Richard Gutteridge at RRes. The report concentrated on the first year field trial, which was carried out at two locations and with natural infection and artificial inoculation treatments. The major conclusion is that there were significant difference in the final levels of take-all between the different sites and treatments. However, overall 2004/5 was a low take-all year. Within the six varieties tested, no significant resistance was found. More detailed studies are required.

Q1. Is the second wheat syndrome only caused by take-all?

Take-all is one of the major causes of the second wheat syndrome, but other contributing factors exist as well. For instance, cyst nematodes may play a role on some fields. Both the sites used for the trails were tested and found to be negative for cereal cyst nematode. Both sites were treated for eyespot.

Q2. Can we rely on your experiments to conclude that no good resistance exists when an elite wheat variety is grown as either a first or second wheat?

The first field experiment was arranged at short notice and therefore the 2nd wheat site is not directly comparable with the 1st wheat site. We need to carry out more studies before we can have a scientific sound judgement

Q3. Should we continue the second wheat syndrome study, or start to work on an alternative trait?

The management team agreed to continue this work for a second field season.

C1. The inoculation method has a strong effect on the overall outcome. Furthermore, different varieties can substantially influence the population build-up of take-all in the soil, which may influence your results. In the WGIN diversity field trial at Rothamsted, the build-up of take-all in the soil has been monitored for both the 1st and 2nd years. These results will be reported at the next WGIN meeting.

A take-all workshop is being arranged and will be held at the University of Cambridge in February 2006 hosted by Prof Chris Gilligan's Group. Please contact Neil if you require additional information.

Follow-up items to be included in the next management meeting

1. Discussion on the new targets for TILLING.
2. More information on the ionising mutagenesis treatment of seeds in China.
3. A report on studies of the straw wall thickness and nitrogen content.
4. The take-all data from RRes.
5. The AxC mapping population data and the Wheat Technology Services offered by RRes should be made available to the public through the WGIN website.

Other points

1. A new WGIN project assistant has been appointed and will start on 19 December 2005.
2. The 3rd Small Grains Cereal Workshop will be held in JIC on 5-7 April 2006. Full details can be found on the BRACT website www.bract.org.

New funding opportunities

1. BBSRC/INRA project. BBSRC will invest £1m for this new initiative. Following on from the joint BBSRC-INRA workshop at Clermont-Ferrand in April, several proposals developed at this workshop were submitted. These included a joint wheat-eyespot and brassica–stem canker proposal (contact Kim Hammond-Kosack), a grain development proposal (contact Peter Shewry) and a nitrogen utilisation efficiency proposal (contact John Snape).
2. Crop Science Initiative. At RRes, the SIFT panel reviewed in total 21 proposals and it was decided that 19 proposals will be submitted. One concern is that very few requests were discussed with the breeders, although the initiative does emphasise the importance of engagement with the industry. The review panel will meet in February and June 2006.
3. LINK projects. Neil Paveley is proposing a LINK project on take-all.
4. Bruno Viegas briefly described the restructuring of Defra pertaining to priority areas and funding allocation. The new policy divisions have been defined. Within the assigned £8m annual research budget, 25% will go to Energy, Waste and Water, 30-40% to Landscape and Environment, a small portion to Climate Change and the remaining to Food. In terms of LINK projects involving wheat research, the proposal submission procedures are as before. More detailed information will be available on Defra website over the coming months. Research concerning “Crop Genetics and Genomics” appears not to fit specifically into any aforementioned specific themes, and cuts across several policy areas. This is because it has elements which actually fit into each of the four new themes. Defra is current organising a review

on programmes of this kind over the next year with a view to commissioning research from April 2007.

5. It was suggested that people should consider Defra as a source of funding when sending proposals to BBSRC-industry partnership programmes for Agrifood community.

The next management meeting will be held on 7 February 2006 at either NIAB or ADAS Boxforth.

30th November 2005

Checked by H-C Jing, KHK, Peter Shewry, Andy Phillips and John Snape