Wheat Genetic Improvement Network

WGIN Stakeholder's Newsletter

April 2006

Next stakeholder workshop: 21st of November 2006 at Rothamsted Research

UPDATE ON PROJECT OBJECTIVES

Project management (Objective 1)
Staff changes: Project assistant Sam Irving left in August 2005 and Sanjay Patel, who was responsible for the website left in October 2005. In December 2005 Elke Anzinger joined as the new project assistant. She is responsible for website maintenance and is assisting with organisational and administrative tasks.

Genetic mapping and marker development (JIC) (Objective 3)
The Avalon x Cadenza doubled haploid population was chosen to be the reference UK wheat mapping population. The aim is to offer to the UK wheat researchers and breeders a map to study QTL and major genes of interest (see previous newsletter for characteristics).

The Avalon x Cadenza map is being developed at John Innes Centre, on the population of 202 validated lines.

Picture 1: *Sampling the Avalon x Cadenza population. John Innes Centre, 2005*

At present, the 202 lines have been genotyped with 129 microsatellites (SSRs) and other types of markers, as well as 251 DArT™ markers (5 markers were removed from the initial list). The resulting map is made up of 90 SSRs, 3 Glutenin markers and 202 DArT™ markers. The map will continue to be developed as more markers and marker types become available.

DArT™ (Diversity Arrays Technology) was created by a multinational research group in Canberra (Australia), Wuhan (China) and Bend (USA) in 2001 (Jaccoud D *et al.*, 2001, Nucleic Acids Research, 29, No.4, e25). This microarray technology gives a genomic representation of DNA of selected wheat varieties around the world. The DNA of these varieties was digested and screened for polymorphisms. The polymorphic fragments have been cloned and then "fixed" on a chip. The companies Diversity Arrays Technology Pty, Ltd and Triticarte Pty, Ltd based in Australia are offering their service to screen any population against this chip.

The Avalon x Cadenza population DNA was sent in August 2005. Within 2 months, the results of this low cost technology completed the SSRs work very efficiently with 202 DArT™ markers mapped. The linkage groups are around 70-120cM, with a marker every 10 to 20cM. The exceptions are with the chromosomes 2D, 3D, 4A, 6D, 7A and 7D, where the linkage groups are smaller. Future work will involve the mapping of more markers in those 6 chromosomes. Different types of markers could be used, such as STM markers which we now have primer sequences for (Hayden MJ *et al.,* 2004, TAG, 108:733-742).The map will be soon available on the WGIN website, as well as some trait data such as ear emergence, height, yield taken over the last 2 years in the field.

Trait identification (Objective 5):
The third year of diversity field trialling to investigate nitrogen uptake and nitrogen use efficiency was successfully sown in October 2005 at RRes. This year's trial contains 24 genotypes, three nitrogen regimes and triple replication.
ADAS and NIAB have sown new trials to continue to investigate 'second wheat syndrome'. The Cambridge field site prepared by growing Soissons last season, was sampled by RRes just after the 2005 harvest and was shown in follow up pot trials to have the potential to cause 30.8% root infection by Take-all on the cv. Hereward.

Exploiting *T. monococcum* as a model for detection of traits, genes and variant alleles and for identifying phenotype:genotype relationships (RRes) (Objective 6)
Einkorn, the common name of *Triticum monococcum* coined from the German expression 'one grain', has been cultivated in many European countries for thousands of years. Although domesticated 10-12 millennia ago together with emmer wheat and barley, einkorn has been rarely exposed to human breeding selection and many landraces and accessions exist. Thus, it is possible to explore the rich variation in multiple traits for genetic improvement of modern bread wheat, which is part of the WGIN core project.

To verify the usage of *T. monococcum* as a model for detection of traits, genes, and novel alleles, it is essential to assess the genetic diversity of *T. monococcum*. For this, a collection of bread wheat A genome microsatellites, so-called SSR (Simple Sequence Repeat) markers, was tested on *T. monococcum* (Figure 1).

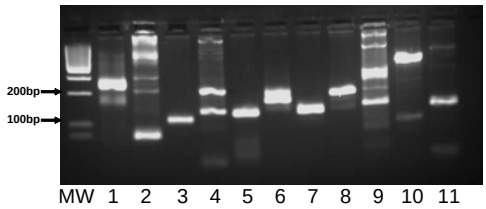

***Figure 1**.: An agarose gel image deciphering the PCR products amplified in Triticum monococcum using primer sets for hexaploid wheat A genome SSR markers. Numbers on the left indicate the sizes of DNA fragments. MW represents DNA ladder; Number 1-11 indicate eleven different SSR markers. The genomic DNA from Accession MDR308 (DV-92) was used as templates for PCR.*

In total, 174 SSR markers have been examined for transferability and it was found that at least 70% of the SSR markers can be applied in *T. monococcum.* Using a subset of 22 SSR markers evenly distributed across the genome, the genetic diversity of 109 *T. monococcum* accessions in the Rothamsted collection was assessed and an overall divergence of 25% found.

One of the key traits examined in *T. monococcum* accessions is their resistance / susceptibility to important UK wheat pathogens. This has been studied in field conditions, glasshouses, or quarantine glasshouses with natural or artificial inoculums. Substantial numbers of accessions showed high resistance (Table1).

Species	Total tested accessions	Resistant
Polymyxa graminis	**124**	**1.6%**
Soil-borne virus (SBCMV)	**124**	**25%**
Septoria tritici	**80**	**100%**
Fusarium	**28**	**7.1%**
***Oculimacula* eyespot**	**27**	**14.8%**

***Table 1**. Resistance/susceptibility phenotyping of Triticum monococcum to important UK wheat pathogens. Accessions resistance to the various pathogens are expressed as the percentage of total number of accessions examined.*

Strikingly, all the tested *T. monococcum* accessions exhibited resistance to *Septoria tritici* leaf blotch, the No.1 disease in UK wheat. In assays performed in controlled environmental conditions, various responses of *T. monococcum* to *Septoria tritici* isolates have been observed, but on all accessions sporulation was blocked (Figure 2).

***Figure 2.** Distinct responses between a Triticum monococcum accession (the 5 green leaves on the left) and the susceptible hexaploid wheat Riband (the 3 yellow leaves on the right) were observed when inoculated with a mixture of nine differential Septoria tritici isolates. The inoculated leaves were photographed 17 days after inoculation.*

We are currently employing a *Septoria* strain constitutively expressing *GFP* (*Green Fluorescence Protein*), combined with light and Scanning Electron Microscope, to examine the infection biology of *Septoria tritici* in *T. monococcum* (Figure 3).

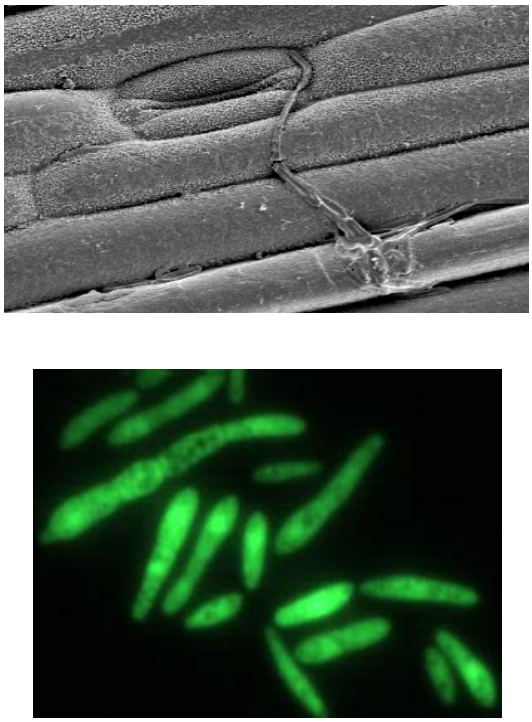

Figure 3. *Tools used to study Septoria infection in Triticum monococcum. Left panel shows an epifluorescence image of Septoria tritici pycnidiospores expressing GFP. Top panel is a Scanning Electron Microscope image of T. monococcum leaf surface inoculated with the GFP strains taken 4 days after inoculation. Note the hyphal growth from pycnidiospore on the leaf surface. The images are taken at Bioimaging Centre at Rothamsted Research with the help from Raffaella Carzaniga and Jean Devonshire.*

Publications

Jing HC, Lovell D, D Kornyukhin, K Kanyuka, K Tearall, A Phillips, S Orford, R Koebner, O.P. Mitrofanova, Hammond-Kosack KE (2005) New approaches for durable disease resistance in wheat. BCPC International Congress & Exhibition – Crop Science & Technology pp 963-970.

Generation of mutagenised populations (RRes and JIC) (Objective 7):

T.monococcum populations. We wish to identify novel variant alleles and then to study specific phenotype:genotype relationships. Currently, three mutagenised populations are under developing. We acquired an EMS population of 1200 M_2 seeds of Accession MDR050 originally created by Kay Denyer at JIC. This population has been grown and leaf tissues used for DNA isolation. A total of 1800 seeds of Accession MDR308 were treated with 0.3, 0.4 and 0.5% EMS, respectively, and the M_1 seeds will be harvested during the period of April to June 2006. Accession MDR308 was chosen because it exhibited good resistance to many UK wheat pathogens and a BAC library is already available for this genotype (Lijavetsky et al., (1999), *Genome **42**:1176-1182.*). A third population has been generated using low energy ion beam radiation platform by our collaborators in China. A total of 5000 MDR308 seeds were treated with three doses of ion beam energy as measured by the number of N^+ ions on a certain area of surface (e.g. $2x10^{16}$ N^+/cm^2). The treated seeds have returned to England and M_0 plants will be grown in the glasshouse at Rothamsted Research.

Identification of gene sequence variants with biological relevance by the PCR-TILLING technique (RRes) (Objective 9)

The TILLING technique identifies mutations in specific genes within large plant populations, highlighting material carrying novel alleles affecting important traits. In order to develop a TILLING resource in wheat, we have taken up to 4,000 EMS treated lines of the spring wheat hexaploid cultivar Cadenza through to the M2 stage. Leaf tissue has been harvested from these plants for genomic DNA extraction and their seed archived. Each of these mutant lines has been sent to the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvasar, Hungary, where they will be grown in head rows for phenotyping and for bulking up seed stocks.

Previously we successfully demonstrated the TILLING technique by identifying a known mutation in an *Rht* gene. We are now optimising the method to allow us to screen for unknown mutations in our initial target genes. The Licor genetic analysers have been established as our mutation detection platform and a move to high throughput TILLING is being made through the installation of a liquid handling robot. Using this system, we are now beginning to detect mutations within our EMS treated wheat population (Figure 4).

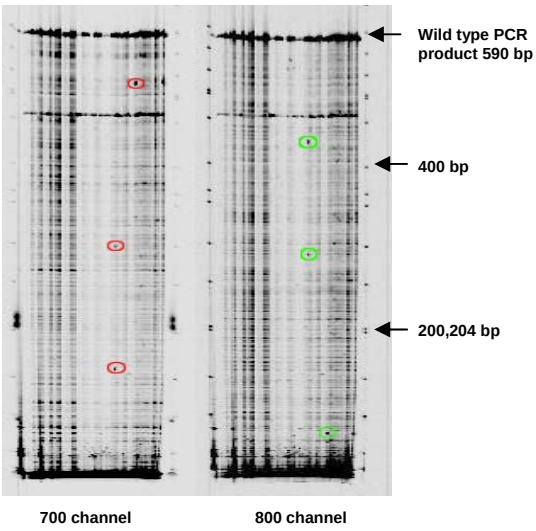

Figure 4: *Mutations detected using the Licor genetic analysers. 96 samples were pooled 2-fold and subjected to PCR with homoeologue specific, fluorescence labelled primers. After melting and re-annealing, the heteroduplexes were digested with the CEL1 enzyme which cuts specifically at base mis-matches. The products were identified by denaturing gel electrophoresis. Three mutations can be seen as cleaved fragments below the wild type band on each image, highlighted in red (left primer) or green (right primer).*

Grain archiving (Objective 11):
From each plot of the WGIN diversity trial 2004/5 1 kg samples of grain have been placed into long term storage at -20C whilst a further 10 kg is being stored at room temperate for one year. Requests for grain samples for any experimental purpose can be made through via the WGIN e-mail address.

Website (Objective 12):
The website is currently undergoing a major update to make it easier to use and more appealing. Several new items have been added, such as a page linking the all the Genetic Improvement Networks (i.e. OREGIN, BEGIN and WGIN), a site map and all the PowerPoint presentations which were given within the framework of WGIN.

Annual Stakeholders Forum (Objective 14):
The meeting minutes and the presentations of the last stakeholder meeting which took place in December 2005 can now be downloaded in the stakeholder section of the WGIN website.
The next WGIN stakeholder meeting will take place on the 21st November 2006 at Rothamsted Research.

Setting up of a LINK project on HFN (RRes) (Objective 16):
Grants by BBSRC (LINK) and Defra (Sustainable Arable Link) and supported by HGCA have permitted the creation of a UK research consortium involving RRes, JIC, University of Nottingham, Harper Adams University College, NIAB and various commercial partners for a 4 year period to investigate pre-harvest sprouting and pre-maturity amylase as factors leading to loss of grain quality, with the aims of understanding the induction and progression of the two syndromes, screening UK germplasm and identifying molecular markers for loci conferring resistance. The project titled "An Integrated Approach to Stabilising HFN in Wheat: Screens, Genes & Understanding" is planned to commence in October 2005. The lead PI is Peter Jack (RAGT), the academic PIs are Andy Phillips (RRes), Michael Holdsworth (Nottingham), John Snape (JIC) and Peter Kettlewell (Harper Adams) and the commercial partners are RAGT Seeds Ltd, Advanta Seeds UK Ltd, CPB Twyford Ltd, Nickerson (UK) Ltd, SW Seed Ltd, Elsoms Seeds Ltd, Biogemma UK ltd, National Association of British and Irish Millers (NABIM), Camden and Chorleywood Food Research Association (CCFRA), Scotch Whisky Research Institute (SWRI) and HGCA.

Publicity (Objective 18):
Several presentations were given at science events in order to raise awareness on the WGIN project (Table 2). The presentation at AAB and the presentation by Kim Hammond-Kosack, Andy Phillips and Neal Evans also introduced other Genetic improvement networks funded by Defra. All the presentations can be accessed through the WGIN website in the Resources section.

Event	Date	Speaker(s)
Association of Applied Biologists (AAB) conference	March 2004	Kim Hammond-Kosack
Science Day - Rothamsted Research Association	June 2004	Peter Shewry
Science Day - Rothamsted Research Association	June 2004	Kim Hammond-Kosack
Biomarket conference	November 2005	Peter Shewry
NIAB	December 2005	Graham King on behalf of KHK
RRes	December 2005	Kim Hammond-Kosack
RRes	December 2005	Kim Hammond-Kosack, Andy Phillips and Neal Evans

Table 2: Presentations at public events

For further information on the WGIN project please see www.wgin.org.uk or contact us at wgin.defra@bbsrc.ac.uk

The contributors to this newsletter were: At Rothamsted Research: Peter Shewry, Andy Phillips, Katie Tearall, Hai-chun Jing and Kim Hammond-Kosack. At the John Innes Centre: John Snape and Leodie Alibert

Next stakeholder workshop: 21st of November 2006 at Rothamsted Research