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

UPDATE ON PROJECT OBJECTIVES

Project management (Objective 1)
Staff changes: Dr. Katie Tearall, who was employed within the WGIN core project at RRes, resigned her post in May to join the Business & Innovation Unit at BBSRC Swindon Office. Katie had successfully implemented the TILLING protocols at RRes and established an EMS-mutagenised population of 4,500 lines of cv. Cadenza. Katie's results indicate a very high mutation rate in this population, which implies a high likelihood of success in TILLING for novel alleles in target genes. We wish Katie all success in her new post. Meanwhile, Carlos Bayon has been appointed as a replacement, as from 18th September. His first task will be to re-establish the TILLING methods and screen the EMS populations for mutations in primary target genes. In April 2006 Dr. Simon Griffiths has taken over the role of Dr. Robert Koebner at JIC.

Germplasm and COS markers at JIC (JIC) (Objective 3)

At the John Innes Centres we were excited to see how our first large scale field sowing of the Paragon EMS population would turn out. Because the lines have now undergone six rounds of single seed descent since mutagenesis they should show beautiful levels of uniformity within rows and fascinating phenotypic differences between rows. This was the case. Some of the more striking mutants were the extreme dwarfs, many late flowering lines, developmental shifts, and loads of the classical phenotypes such as awns, loss of wax, club ears ... This genetically fixed population has immense value for UK wheat research and is now being exploited in a number of projects including the BBSRC/INRA nitrogen use efficiency project.


Field trial of the mutant Paragon collection, JIC

Also at the JIC Church Farm we grew the Watkins collection (3200 lines) and the Defra reference mapping population Avalon x Cadenza. The aim of the study of the Watkins material was to assess the heterogeneity of the accessions held at JIC, so four head rows were grown for each. The material from each row will now become a distinct accession. The Watkins collection was also phenotyped for height (with plant growth regulator), flowering time, and vernalization requirement. The use of a collection like the Watkins is an altogether more daunting task than the conceptually simple experiments possible with the Paragon EMS population. It is interesting to note though that a subset of the Watkins sent to Australia in the 1980s turns out to be the source of some important nematode resistance for Australian wheat breeding programs. It can be done and the rewards are potentially great.

A great resource for mapping, genotyping, and mutation detection in all these germplasm resources are Conserved

Orthologous Sequence (COS) markers. With primers anchored in exons these markers are highly transferable between grass genera but because they amplify across introns (highly mutated compared to exons) the products are rich in SNPs. The problem for wheat has always been finding a high throughput platform to resolve the SNPs. We now have these markers working as well as SSRs on an ABI 3730. Our first set of 140 COS primers designed to give even coverage over the wheat genome will soon be posted on the WGIN website. We will design and test more, mapping them on the Avalon X Cadenza double haploid population. Please contact Simon Griffiths (simon.griffiths@bbsrc.ac.uk) if you would like to become involved with this work or use any of the resources mentioned.

Exploiting *T. monococcum* as a model for detection of traits, genes and variant alleles and for identifying phenotype: genotype relationships (RRes) (Objective 6)

To achieve these aims, a global collection of *T. monococcum* accessions was obtained. In the previous newsletter (February 06), we reported that a considerable genetic diversity exists in the RRes *T. monococcum* collection as assessed by a type of molecular markers called SSR (Simple Sequence Repeat). This provides a basis for phenotype/genotype association studies.

We selected 30 *T. monococcum* accessions and examined the variation of several important morphological and agronomic traits including plant height, number of tillers, yield, grain storage protein profiles, grain hardness and resistance to abiotic and biotic stresses. Several traits have been found to be associated with SSR markers located at specific chromosomal regions. For instance, a large variation in grain hardness was observed in the 30 *T. monococcum* accessions and this variation was linked to the genetic differences in a region on chromosome 5A which encompasses the *Hardness* locus.

Another activity pertaining to identifying phenotype:genotype relationships is the construction of a high-resolution SSR marker map for *T. monococcum*. For this, we generated mapping populations by crossing accessions with contrasting phenotypes in key traits. A F_3 population consisting of 94 individuals from the cross MDR308 (DV92) x MDR002 has been used for the construction of a SSR marker linkage map. Li-cor slab-gel based DNA analyser and fluorescence dye-labelled primers are used for high throughput multiplexing SSR marker analysis (Figure 1).

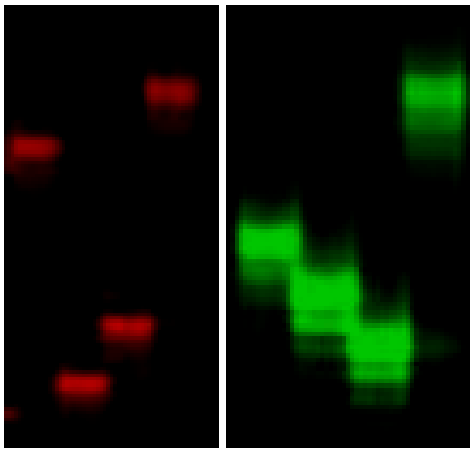

***Figure 1**. Constructing the first T. monococcum SSR marker map. Two Li-cor gel images deciphering the PCR products amplified in Triticum monococcum and T. aestivum using red and green fluorescence-labelled primer sets from hexaploid wheat A genome SSR markers. The four red and green bands in the two images represent SSR markers amplified from Avalon, MDR308, MDR002 and Cadenza from left to right.*

Once constructed, the linkage map will facilitate association genetics for the identification of novel genes and variant alleles. So far we have assigned over 60 SSR markers on the 7 chromosomes. These SSR markers are also located on the A genome of *T. aestivum*.

Our three-year of field experiment indicates that all the tested *T. monococcum* accessions exhibit high resistance to *Septoria tritici* (telemorph *Mycosphaerella graminicola*) leaf blotch under UK wheat production conditions (Figure 2).

***Figure 2.** High resistance of T. monococcum to Septoria tritici blotch under UK wheat production conditions. The bread wheat Hereward (left panel) has high numbers of leaf lesions, whereas in the Triticum monococcum accession (right panel) no leaf lesions were observed. No fungicides had been applied to the trial. Photos were taken in June 2006.*

A detailed cytological study indicated that the post-penetration hyphal growth of the fungus was reduced in most of the *T. monococcum* accessions (Figure 3).

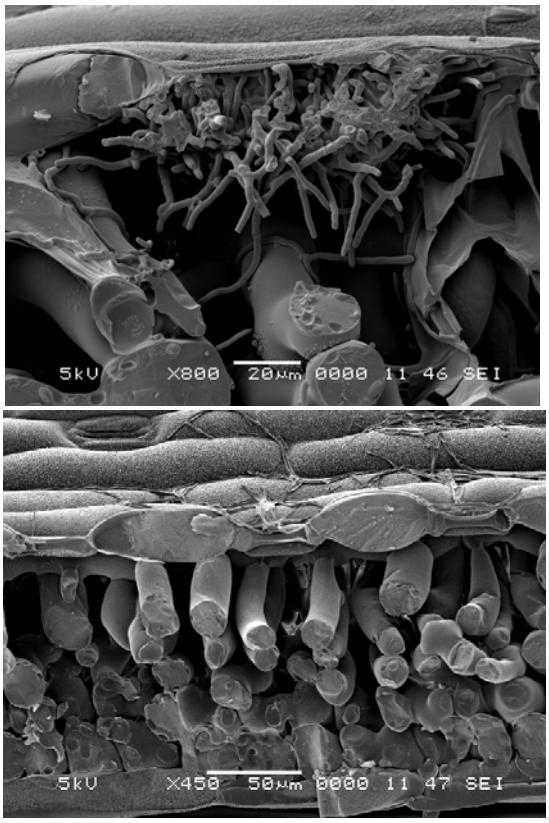

Figure 3. *Scanning Electron Microscope Cryofracture images showing different growth patterns of Mycosphaerella graminicola (Septoria tritici) in leaves of bread wheat Riband (top panel) and T. monococcum accession MDR308 (bottom panel). Abundant post-stomatal growth of Mycosphaerella graminicola was observed in bread wheat, whereas in most of T. monococcum accessions fungal hyphae mostly grew on the leaf surface but post-stomatal growth was restricted. The images were taken 11 and 24 days post inoculation for Riband and MDR308, respectively, at the Rothamsted Research Bioimaging Centre (courtesy of Jean Devonshire).*

Under controlled environmental conditions by varying light and humidity, sporulation could be triggered for a few *Septoria tritici* isolates on a limited number of *T. monococcum* accessions. Thus, both field and controlled environmental tests indicated that *T. monococcum* could be a rich source of resistance to *Septoria tritici* leaf blotch. More importantly, the identification of both resistant and susceptible *T. monococcum* accessions has allowed us to study the mode of inheritance of resistance in *T. monococcum.* This genetic dissection of resistance is underway using the F_3 population from the cross MDR308 (DV92) x MDR002 which is also used to construct the SSR map as mentioned above.

To demonstrate the usefulness of the resistance traits for bread wheat genetic improvement, we selected two *T. monococcum* accessions (MDR308 and MDR002) and crossed them with genotype Chinese Spring and two UK wheat cultivars, Riband and Cadenza. Over a dozen viable hybrids have been obtained through either directing seed setting or embryo rescue (Figure 4). These will be backcrossed to a small selection of bread wheat cultivars in the near future.

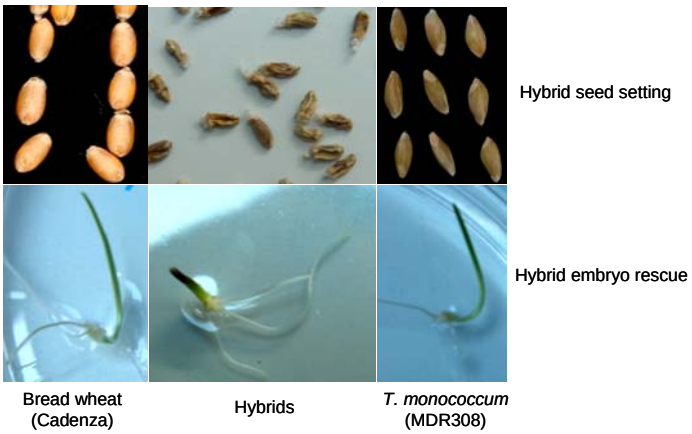

Figure 4. *Comparison of morphology and growth status of T. monococcum and bread wheat hybrids with corresponding parental lines. The male organs of bread wheat varieties were emasculated and pollinated with pollen from T. monococcum donors. The top panel shows that the hybrid seeds obtained by letting the crosses set seed are often shrunken and small in size in comparison with the parental lines. The bottom panel shows the growth of the hybrid seedlings obtained through embryo rescue 19-21 days after pollination and the corresponding parental lines. The embryo rescue work was carried out at the RRes Wheat Transfor*mation *Lab (Angela Doherty and Huw Jones).*

A forward genetics approach has been employed in WGIN objective 6 to generate mutagenised *T. monococcum* populations for detection of novel variant alleles. In total 1500 M_2 seeds of accession M308 (DV92) treated with 0.3, 0.4, or 0.5% EMS were obtained. Individual lines comprising the M_3 population will be multiplied at the Agricultural Research Institute of the Hungarian Academy of Science at Martonvásár, Hungary over the 2006/2007 field season. It is anticipated that field phenotyping will be made at several times during the growing

season. Accession M308 (DV92) was chosen because it exhibited good resistance to many UK wheat pathogens and a BAC library is available for this genotype (*Lijavetsky et al., 1999, Genome* ***42****:1176-1182.*). A third mutagenised population generated through the use of a low energy ion beam radiation platform by our collaborators in China was sown in the RRes glasshouse and M_2 population is expected to be harvested in early 2007.

Collectively, these three *T. monococcum* mutagenised populations will form the reverse functional genomic platform and will be used for novel allele mining by the TILLING technique. The gene *RAR1,* coding for one of the important signalling components in plant defence responses, has been selected as the initial TILLING target gene. The *RAR1* gene has been identified in *T. monococcum* and the three homoeologous genes in bread wheat have also been identified. Currently, we are using TILLING to identify natural and induced variant alleles and examine the role of *RAR1* in wheat resistance to major UK wheat pathogens.

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: Andy Phillips, Hai-Chun Jing and Kim Hammond-Kosack. At the John Innes Centre: John Snape, Leodie Alibert, Simon Griffiths and Simon Orford.

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