Next stakeholder workshop: 14 November 2008 at Rothamsted Research

Defra Wheat Genetic Improvement Network – The Core Research Project

Background

The UK government is committed to a more sustainable agriculture. Wheat is grown on a larger area and is more valuable than any other arable crop in the UK. The overall aim of this project is to generate pre-breeding material carrying novel traits to the UK breeding companies and to deliver accessible technologies thereby ensuring the means are available to produce new, improved varieties. An integrated scientific 'core' which combines underpinning molecular markers, genetic and genomic research, together with novel trait identification, are being pursued to achieve this goal. The programme is managed by a team including representatives of the key UK research groups and breeders. They ensure the programme and its outputs are communicated to the wider scientific and end user communities, via a web site, a stakeholder forum, focused meetings and peer reviewed publications. The WGIN collaborates with equivalent operations overseas to ensure the programme is internationally competitive.

This project

The Core Project started in 2003 provides genetic and molecular resources for research in the Satellite Defra Projects and for a wide range of wheat research projects in the UK. These resources include wheat genetic stocks, mapping populations, molecular markers and marker technologies, trait identification and evaluation, genomics and bioinformatics. The Research Platform will promote the integration of the funded work.

Assessment of the A.E. Watkins wheat collection in 2008 for resistance to foliar, stem base and root diseases Objectives 2 and 5

Take-all, caused by the fungus *Gaeumannomyces graminis var. tritici*, is consistently the most damaging root disease of wheat and barley in the U.K. Symptoms of the disease appear as black necrotic lesions on the roots and when severe causes the plant to die prematurely (Figure1 overleaf). Yield losses vary and can range from 5% to over 60%. This disease is usually only a problem when two or more susceptible cereal crops are grown consecutively, but in 2008 there have been more reported cases of severe take-all in first wheat crops. In the absence of an actively growing host plant, the take-all fungus survives between crops on infected root debris which is the main source of inoculum for a following susceptible crop. However, in this saprophytic phase the fungus competes poorly with other soil microbes and the infective inoculum declines rapidly. Due to this lack of soil survival, a one-year break is normally sufficient to provide effective control of the disease.

In 1987, two thirds of the wheat acreage in the U.K. was grown either as a second or more consecutive crop. By 1996, as break-crops became more profitable, this had decreased to 26%. Two years later, probably as a result of the gradual withdrawal of subsidies, the proportion of crops at risk from take-all had increased to 41%. In 2008, following the withdrawal of one year set-aside, the proportion of crops at risk from the disease is likely to be the same, if not higher, than in the mid 1980's i.e. > 66%. A rotation of break, wheat, wheat is common practice but an even shorter rotation of break-wheat has been introduced on some farms.

Figure 1. Necrotic lesions on wheat roots caused by *Gaeumannomyces graminis var. tritic*

Fungicides, applied as a seed dressing or foliar spray in spring, have the potential to reduce the severity of the disease but do not eliminate the disease problem. Over the past 50 years there has been little advancement in breeding for resistance to take-all. However, some promising lines from the diploid wheat species *Triticum monococcum* have been identified (WGIN newsletter October 2007) and may prove useful for future breeding programmes. Another source of breeding material may arise from the 1930's wheat collection of A.E. Watkins which is being evaluated at Rothamsted and the John Innes Centre for various traits.

The A.E. Watkins wheat collection, recently resurrected at JIC (WGIN newsletter May 2008), was sown on Long Hoos field at Rothamsted on October 23rd 2007. Assessment of take-all on samples taken from the previous wheat crop on this site showed moderate infection, therefore, the soil inoculum level was likely to be high and pose a threat to the following wheat crop. The experiment was hand sown, and each plot consisted of 3 rows each 50 cm in length with a 50 cm unsown path surround. The experiment contained 740 Watkins lines and 5 block replicates each with eight controls. The eight controls were oats, rye, triticale and 5 other hexaploid wheats. A further 20 plots of the highly susceptible variety, Hereward, were included in an Alpha design. The main aim of the experiment was to evaluate the collection for any possible resistance to root and stem base diseases and assessment of foliar diseases provided additional information. To encourage the build up of foliar diseases no fungicides were applied but three doses of growth regulator were used to reduce crop height. The rest of the field was sown to the winter wheat cv. Brompton and the 12 m which surrounded the experiment received no fungicide. Some slight septoria leaf blotch infections were seen in December and the first record of mildew and yellow rust were on May 6th and 13th, respectively. On May 22nd growth stages ranged from 37 – 49 (flag leaf visible to early ear emergence). The crop was inspected on June 6th where yellow and brown rust, septoria and mildew were found to be common. The whole experiment was assessed for these foliar diseases between June 16th - 20th and scored, based on the degree of infection on a plot basis, on a 5 point scale. The rusts, yellow and brown, were either absent or occurring in trace amounts on the control varieties. Septoria and mildew was more common but generally only seen on the lower leaves, except on Riband, where septoria was present on some flag leaves. The Watkins collection showed a range of infection from resistant to fully susceptible for each of the diseases. The number of lines in each category for each disease is

shown in Figure 2. This experiment was proving to be a pathologist's paradise and the site was visited by the WGIN management group on June 20th. The group also visited the neighboring *Triticum monococcum* / hexaploid wheat trial where foliar diseases were at a similar level on

The winter wheat surrounds were assessed for take-all and eyespot in April, May and June. The percentage of plants with take-all doubled between April and May from 35% to 70%, and the severity of disease in June indicated an overall moderate attack of take-all. Eyespot was

Figure 2: Susceptibility of Watkins accessions to foliar disease

the hexaploid wheats. However, on the *T. monococcum* lines no foliar diseases were seen. Collating all the hexaploid data revealed that only 16 lines (2%) were fully resistant to septoria. But of these, 12 were also fully resistant to the other three foliar diseases (table 1). The entire foliar dataset is available on the WGIN website in Excel format.

common and by May 98% of plants and 70% of straws were infected.

All plots were sampled at the beginning of July to acquire root/stem base samples for disease assessments. Plants from three 20cm length of row were dug up from each plot and samples washed, air dried and stored for root and stem base disease at a later date (Figure 3A overleaf - polytunnel).

Disease	Total number of resistant accessions
All four foliar diseases	12
Septoria only	16
Yellow and Brown rust and mildew only	58
Yellow and Brown rust only	139

Table1: Watkins accessions - resistant phenotypes with nil disease rating

Severe take-all was clearly visible on some of the washed samples (Figure 3B).

Figure 3A: Air drying of root samples

Figure 3B: Take-all on washed root samples (left: severe, right: moderate)

Investigating the control of ear emergence in UK winter wheat Objective 3

Why is ear emergence important?

Ear emergence in wheat is a complex trait which has significant effects on crop performance in terms of yield, stress tolerance, disease avoidance, resource use, farm management and grain quality. A lot is now understood about the major ear emergence genes. These genes have such large effect that their presence or absence actually changes the agronomic type of the variety in question. The best examples of these effects are the *Vrn* genes which determine whether a variety is a spring or winter type, and the *Ppd* genes which control the response of wheat to day length. Figure 4 depicts the segregation of the *VrnA* promoter sequence in the Avalon x Cadenza population.

In the UK we mostly grow winter wheat varieties that require the increasing day length that marks the onset of spring in order to flower. This means that the major ear emergence genes are not usually variable in UK wheat. In spite of this, the families that plant breeders develop from UK wheat crosses, show lots of variation in ear emergence time. The range of ear emergence times is typically five to ten days, and from these individuals come the varieties that appear on The Recommended List. It is important to know whether the different genes controlling flowering time are actually contributing to the improved crop performance of new varieties, or whether they are simply passengers that add unnecessary complexity to the breeding process.

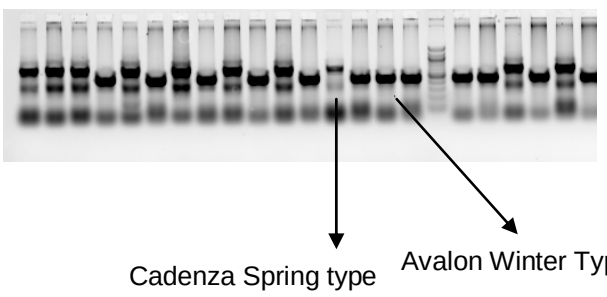

Figure 4: Segregation of *VrnA* (promoter sequence) in the Avalon x Cadenza population

The WGIN population

The Avalon x Cadenza mapping population, developed under WGIN, is a useful resource for addressing this question. Avalon is a winter wheat bred at the Plant Breeding Institute from a cross between Maris Ploughman and Bilbo. Cadenza is alternative wheat, which means that it does not require the prolonged cold of winter to induce ear emergence, but it does perform well as a winter or spring sown crop. Cadenza was bred by Cambridge Plant Breeders from a cross between Axona and Tonic.

Finding the genes

Ear emergence is controlled by several genes. To identify individual gene effects in a family of wheat lines (better referred to as a population) the parental makeup of each chromosome needs to be established. The progeny of Avalon and Cadenza have chromosomes that are a composite from each parent. If we focus on a particular chromosomal region then half of the individuals in the family will have the Cadenza type and the other half will have the Avalon type. So for this population there will be ~100 individuals in each group. If the average ear emergence time for each group is the same the conclusion can be drawn that there are no genes influencing this trait in this experiment. However, a statistically significant difference between the two groups indicates that there are ear emergence genes on that chromosomal segment.

This type of study is referred to as Quantitative Trait Locus (QTL) analysis. It requires knowledge of the chromosomal composition (genotype) for each individual in a population and the trait data, in this case ear emergence time, for each of those individuals. These data were collected for the Avalon x Cadenza population as part of the WGIN programme. The QTLs identified were compared to those identified in three other crosses: Spark x Rialto, Savannah x Rialto, and Charger x Badger. QTL locations were then compared between maps in a meta analysis. A summary of that analysis is shown in Figure 5.

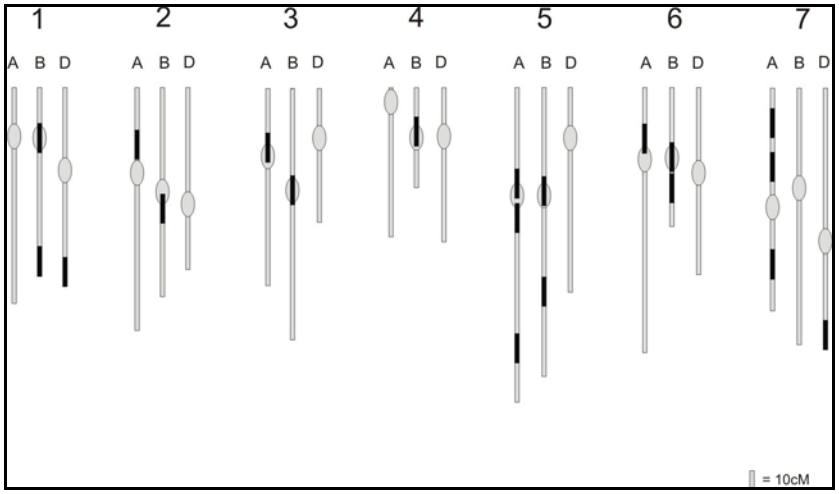

Figure 5: Summary of QTL meta analysis: Wheat has 21 pairs of chromosomes (1A, 1B, 1D, 2A etc through to 7D). The sticks represent these chromosomes and the ovals a functional part of a chromosome called a centromere. The estimated location of genes are QTL shown as black bars.

These QTL explain a very high proportion of the variation in ear emergence found in these crosses. In turn, the parents of the crosses represent a wide range of UK winter wheat germplasm. Knowledge of the location of these genes will help plant breeders to explain and predict variation in ear emergence at the molecular level.

The WGIN programme has collected a wide range of data for numerous traits in Avalon x Cadenza including yield potential, nitrogen use, biomass and height. Figure 6 overleaf shows the 2008 Avalon x Cadenza field trial at JIC. A similar analysis to that described for ear emergence will be carried out to build a better understanding of how well UK winter wheat is equipped to deal with future pressures and needs. This will help us to target the variation there is more precisely and to search for new and useful genetic variation.

Figure 6: JIC Avalon x Cadenza field trial in Norfolk, picture taken in summer 2008

Developments in Mutagenesis and TILLING Objective 9

The hexaploid nature of bread wheat limits the utility of mutagenesis as a tool for crop improvement as recessive mutations in individual genes are likely to be complemented by functional homoeologous copies. However, various technologies are now available that permit the identification of mutations in specific target genes, allowing the potential of these genes for crop improvement to be explored. With this in mind, we produced a mutagenised population in the spring wheat variety Cadenza by ethylmethane sulphonate treatment of seeds. The final population, archived as M_3 grain, numbers some 4,200 lines. Despite a high mutation rate – we estimate that each M_3 individual carries more than 300,000 point mutations – most of the lines are fully fertile and obvious phenotypes are relatively rare (<5%), although measurement of a range of characters by our collaborators at Martonvásár in Hungary identified quantitative phenotypes in over half the lines.

The TILLING methodology, originally developed by the University of Washington in Seattle, permits the identification of single nucleotide polymorphisms and point mutations within germplasm collections and mutagenised populations. The technique relies on PCR with gene-specific primers followed by cleavage of heteroduplexes with a mismatch-specific nuclease. Pooling of DNA samples up to eight-fold and analysis of large target regions (up to 2 kbp) allows high throughput, but we have found that, in practise, lower rates of detection are typical in wheat, as the large genome and high GC content contribute to difficult PCR conditions. The method is also relatively expensive, as it involves the use of labelled primers and several labour-intensive steps including gel electrophoresis. Although false positives are rare as both strands of the PCR product yield signals, providing confirmation of putative mutants (see Figure 7 overleaf), difficult targets often have a high false negative rate. Nevertheless, we have identified more than 200 point mutations in target genes involved in hormone signalling and starch biosynthesis using this method.

We have recently developed an alternative strategy for mutation discovery that relies on the detection of changes in the melting properties of the heteroduplex DNA formed after annealing of wild-type and mutant PCR products. The Lightscanner instrument detects temperature-dependent loss of fluorescence from the dsDNA-specific dye, LCGreen (Figure 8 overleaf), as the sample temperature rises. Although the target region is limited in size to less than about 300bp, the method is very sensitive and has a very low failure rate. This method is particularly efficient for target genes that contain small exons interspersed with larger introns, as the screening can be restricted to exons and

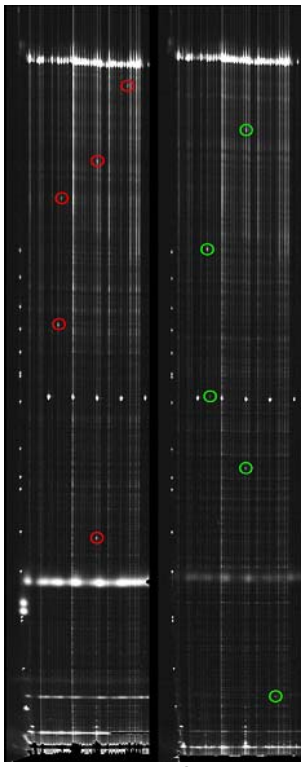

Figure 7: TILLING for mutations in GA20ox1A. Two-fold pooled DNA from mutagenised M_2 plants. Green circles show hits in the sense strand, red shows the corresponding mutations in the complementary strand.

their adjacent splice sites. Although unlabelled primers are used, the smaller target region and the expense of the fluorescent dye keep costs similar to TILLING.

Recent advances in DNA sequencing suggest an alternative strategy for mutation discovery. The latest generation of high-throughput sequencers will be able to generate up to 400 million base pairs per run; thus, re-sequencing of target genes within a mutagenised population is a realistic proposition. In collaboration with the University of Liverpool, we are currently investigating multi-dimensional pooling strategies that will allow us to track thousands of individual DNA samples through amplification and sequencing. One advantage of this approach is that it may not be necessary to develop homoeologue-specific PCR primers for the target genes, as each mutation can be assigned to the A, B or D genome based on linked polymorphisms. In addition, no dedicated equipment is required as the high-throughput sequencing required is likely to be subcontracted to a specialist provider. The costs of this method have yet to be determined, and will depend on the over-sampling required to identify all possible mutants in an amplicon pool.

The development of multiple platforms for mutation detection should thus increase access for scientists and breeders to the wide range of genetic variation available in natural and mutagenised populations.

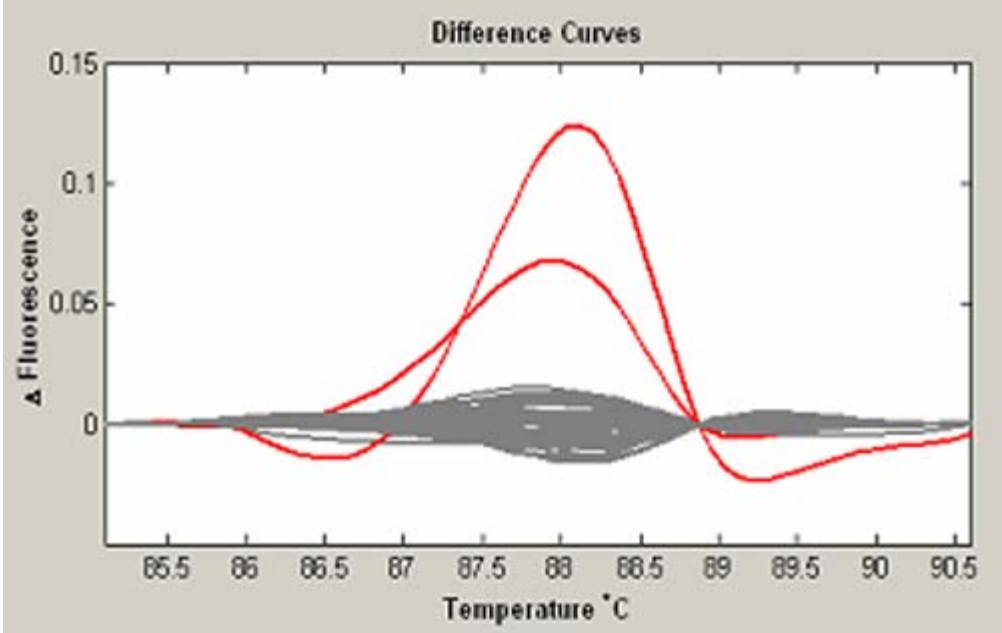

Figure 8: High-resolution melt analysis for mutation screening in GA20ox1D. Two-fold pooled DNA samples from mutagenised M_2 plants were amplified by PCR, annealed in the presence of LCGreen and melted in the Lightscanner. The figure shows a difference plot of fluorescence against temperature. Wild-type amplicons are shown in grey, putative mutants in red.

However, the major limitations to full application of this technology remain our limited knowledge of genes involved in important traits, which restricts our choice of target genes, and the lack of full genome sequence in wheat, which slows the development of the targets, irrespective of the detection platform.

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

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