

## WGIN Management Meeting – 6<sup>th</sup> July 2004, John Innes Centre

### Attending:-

#### Management Team

Robert Koebner, John Snape, Andy Phillips, Kim Hammond-Kosack, Bruno Viegas, Thomas Jolliffe, Rosemary Bayles, John Foulkes, Neil Paveley, Peter Jack, Chris Chapman, Peter Shewry

#### Observers

Katie Tearall, Hai-Chun Jing, Pauline Stephenson, Simon Oreford, Michelle Leverington, Yingkun Wang, Matteo Clavarrolla, Steve Reader, Mike Ambrose, Mary Byrne, James Simmonds, Leodie Alibert

### Report on year one progress and research goals

#### Robert Koebner

##### Objective 2

Watkins Collection - 900 lines purified by individually bagging 4 plants/line. Also squashed leaf DNA onto FTA card for PCR genotyping. Noted variation in height, flowering time, mildew resistance. Plants grown in glasshouse and treated with PGR.

EMS mutagenesis (3hr, 1%). 3,500 plants of spring cv Paragon grown in glasshouse. At least one ear per plant bagged to ensure selfing. Limited variation observed, eg some sterility. Will take through SSD. Aim to produce 5,000 lines.

##### Objective 3

Avalon x Cadenza selected as core mapping population. Approx 1/3rd of population (204 lines total) already partially mapped. Identified SSRs that are informative for full mapping in year 2.

SNP development - Tried to identify SNPs electronically from databases but failed, probably due to low polymorphism and presence of errors in EST databases. Now looking at published and in-house genomic sequences. So far less than 10 SNPs developed. Focus on SNPs within genes to complement microsatellite markers. Included *Pin* gene SNPs. The PIN gene encodes the puroindoline family that confer grain hardness.

STMP (sequence tagged microsatellite profiling) - Over 300 loci identified and mapped on all 42 chromosomes as part of collaboration with Value Added CRC in Australia. Observed highest level of polymorphism in B genome. Will be used for mapping in the Avalon x Cadenza cross.

Functional multilocus DNA fingerprinting - NBS genotyping/Gediflux set - collected 40,000 datapoints on Gediflux set of 500 European post 1940s winter wheats. Not mapped.

Mapping ESTs to BACs – not yet started.

#### Objective 4

GAIT database available via internet (<http://jic-bioinfo.bbsrc.ac.uk/cereals/Gait.html>)

Gediflux (EU) - One SSR per chromosome arm for all 500 + entries (file to be available on WGIN website). NBS profiling – 3 primers x 3 REs completed on  $\approx$  50 loci. Transposon-based fingerprinting (SSAP)  $\approx$  70 loci. Gediflux has shown no real evidence for narrowing of diversity in European winter wheat germplasm over last 50 years.

#### **Questions Taken:-**

Do you store the tissues prior to DNA extraction using FTA cards?

No plant tissues samples stored, only fresh leaves were punched and used for DNA extraction.

Did you score the phenotypes observed such as albino and chlorotic lesions in your EMS mutagenised population?

No, it was only observation, no real scoring data.

In total, 3500 EMS mutagenised lines were generated, are you planning to do mutagenesis again since the target lines should amount to 5000?

No, since the Watkins collection together with EMS lines already exceed 7000.

Do you have any particular interesting genes to focus on, why were SSRs chosen to do physical mapping?

Yes, for instance, NBS class of disease resistance genes. Up to date, 335 loci identified for STMP, a technology developed in collaboration with University of Sydney.

Are you able to use STMP on several platforms?

We use fluorescence-based assays (ABI platform), but standard silver staining (or radioactive labelling) are feasible.

Mapping ESTs to BACs has not yet started – this may require additional financial support from JIC.

What is the SSR for? How can they be used for breeding? Do you have a complete set of markers?

Yes, it is possible for breeding purpose. One of the results comes out from the SSR mapping study is that the conclusion which the genetic diversity among modern hexaploid wheat varieties is narrowing, is wrong.

Are there any phenotypes or traits linked to the 500 SSR entries?

None identified to date.

**Kim Hammond-Kosack**

## Objective 6

KHK reported on work carried out at RRes on the diploid wheat *Triticum monococcum* representing the AA genome of the bread wheat. In total 124 accessions have been collected worldwide and their resistance to major British wheat pathogens has been examined including soil borne cereal mosaic virus (SBCMV) and its vector *Polymyxa graminis*, *Septoria tritici* leaf blotch, (*Mycosphaeella*), *Tapesia* eyespots, *Fusarium* leaf blight. All the examined accessions are susceptible to *Polymyxa graminis*, but exhibited variations in the resistance to SBCMV. The accessions also showed a high degree of resistance to *Septoria*.

## Questions

Can *Septoria tritici* infect *T. monococcum*? Yes Koch's postulates confirmed in single isolate glasshouse inoculations.

Are you planning to test the diploid accessions with non UK isolates? Yes, a differential set of non-UK origin has recently been obtained from Prof. Gert Kema Lab (The Netherlands).

The second part of KHK's report was on PCR TILLING of key wheat defence signalling regulators. Current knowledge indicates that NPR1 (non expressor of *PR* genes), RAR1 (required for Mla-mediated resistance) and its interacting components SGT1 (Suppressor of G2 transition), are the three key components in mediating plant resistance signalling in multiple plant species. . Hence, they were chosen as the PCR TILLING targets. *In silico* study recovered wheat homologues of the three genes in EST databases, and their identities have been studied at the molecular level. The CODDLE programme is being used to predict genic regions suitable for TILLING. The ultimate goal of this study is to establish novel link between variant alleles of these genes and the resistance phenotypes in *T. nonococcum* accessions.

## Questions

KHK was asked by the breeders why only these three genes were selected, and if no clear correlations found, whether there are backups. Yes backup candidate genes for plant defence are available.

KHK was also asked to explain how CODDLE programme predicts suitable TILLING regions. CODDLE examines the potential of GC to AT transitions caused by the mutagen to lead to changes in amino acid sequence that would affect protein function as predicted from protein sequence alignments.

## **Andy Phillips**

## Objective 9

Andy Phillips reported on work being carried out at RRes on Objective 9 – TILLING in hexaploid wheat. The Reduced height gene (*Rht3*) will be used as a proof of concept for TILLING as it is a semi-dominant trait. RHT B and D genome specific primers have been designed. These primer pairs are different from, but based upon those in Ellis *et al.*, 2002. For high throughput TILLING a pool depth of between 4

and 8 samples has been suggested but this is dependent on the PCR background, primer specificity etc and will have to be investigated. EMS treated Rht3 Mercia has been sown in the field. EMS treatments were done at 3 different EMS concentrations (0.3 %, 0.6 % and 1.2 % EMS). A range of EMS concentrations were used rather than a single concentration to generate populations with differing numbers of mutations. It is also crucial to have a good mutagenised population in order to perform efficient PCR TILLING.

### **Pedigree of Avalon and Cadenza**

Pauline Stephenson and Thomas Jolliffe

Reported Cadenza and Avalon pedigrees – to place on WGIN website ([File name](#)) . Cadenza includes Sanova (Mexican) via Tonic.

### **Genetic Stock at JIC**

Mike Ambrose

Mike Ambrose described JIC Public Cereal Collections, which consist of BBSRC collection, wild barley collection, bread wheat collection, Durum types, Watkin collection, and precise genetic stocks. A web-based database is available for users to search. <http://www.jic.ac.uk/GERMPLAS/Index.htm> and <http://data.jic.bbsrc.ac.uk/cgi-bin/germplasm/cereals.asp>

Public Grain Collection databases - 9,400 wheat lines.

Commercial	2,353*	*includes BSPB NLT1 lines
Include commercial UK	700	
Breeders' lines	2,208	
Land races	3,658	
Durum cv	89	
Durum land races	49	

### **WGIN website**

Kim Hammond-Kosack

The WGIN website is now live ([www.wgin.org.uk](http://www.wgin.org.uk)). Comments and suggestions for the new website were asked for. Presentations given at the WGIN TRAITS meeting (10 June 04) will be posted on the website if individual speakers agree. PS suggested that the 'facilities' button should be changed to 'resources'. In the 'contacts' section it should be made clear whether this is contacts outside WGIN or within the WGIN team itself. The website will be made international in year 2 with links to overseas projects and organisations. As well as having links to other sites, it may be beneficial to analyse the latest activities of these web sites via a hot topics section on the first page. This could also be achieved using a bulletin board or via emails informing subscribers of recent activities etc. Could there be links between DEFRA and WGIN latest announcements? BViegas to look into this. (**Action item**).

Suggestion for improving the website

- Facilities change to resources
- Contacts change to links

- Meeting is not precise
- International links to be added
- Hot topics on the first page?
- E-bulletin
- Defra website to put forward WGIN announcement

### **Report from TRAITS meeting June 10<sup>th</sup> 2004**

Neil Paveley

It was previously suggested that traits investigated within the WGIN core project may vary from year to year, depending on agreed priorities. Important traits and their priority level were discussed at the TRAITS meeting. NP reported the following as having been identified as important traits, listed in order of decreasing priority by the plant breeders.

1. Hagberg/sprouting  
LINK proposal in preparation by JIC/RRes/Nottingham. May also be BBSRC proposal.
2. Septoria  
Adequately covered by current research.
3. Second wheat syndrome  
Difficult to study, no obvious and feasible leads. May be appropriate for scoping study. Take all is an important component, but there are probably other components.
4. Orange blossom midge  
Very important but regional. Current LINK project may be renewed.
5. Lodging – feasible, breedable, covered by current breeding programmes and LINK.
6. BYDV – feasible, little work, possible target. Scoping study in place led by ADAS. Interest by John Pickett's group at RRes.
7. Other insect pests  
Moderate importance, feasible.
8. N use efficiency  
Low priority for breeders. More important for sustainability. Needs “market pull”. New ADAS/Syngenta LINK project on low N feed wheat called ‘Green Grains’.
9. Need to maintain skills base in field pathology/pest biology.

It was asked whether the presentations in the traits meeting can be viewed, and whether these traits studies can be performed in scoping studies or put as LINK projects.

How can all these traits studied be successfully anchored to WGIN?

### **Summary of other DEFRA funded wheat projects**

Kim Hammond-Kosack

BV performed a search for projects funded by Defra throughout history. In total well over 100 projects were recovered. Breeders asked if it was possible to obtain DEFRA final reports. In response, BV said that for projects completed after 1998 the final

report will be made available on the Defra website. For older projects, and those completed with no final report on the website, Defra will provide final reports on request

How much is the funding from Defra for wheat annually?

It is difficult to provide a figure for all research specifically on wheat, since many projects cover also other crops. As a very rough estimate, I would suggest at least £2 million per annum. After the meeting, I checked and for projects including wheat research (excluding environmental protection), the total was £5m in 2003/04. I attach a list of the projects below.

### **Field trial 2004/2005**

Kim Hammond-Kosack

An overview of the proposed nitrogen use efficiency (NUE) field trial was presented by KHK. The experiment will involve the evaluation of 30 hexaploid wheat genotypes, primarily class 1 and class 2 bread wheat that are diverse in both genome and genetic origin, with the aim of identifying genetic diversity in NUE. An N treatment of 30 wheat varieties at 100 N and 12 selected varieties at 200 N was proposed. The breeders asked what measure of nitrogen NUE would be used. Soil mineral N, N uptake measurements in the shoots at anthesis (by destructive sampling) and N uptake in straw and grain were proposed. The breeders consider that NUE is of limited commercial value and is unlikely to be incorporated into their breeding plan. Breeders feel that exotic germ plasm will be used in the trial and this cannot be expected to perform well under UK conditions. In response RRes stated that genetic diversity in NUE, grain quality and canopy architecture would be analysed and are of considerable importance. The breeders were unhappy with the N application rates suggesting that 200 N should be used for all the wheat varieties as 100 N would not be used in the field. In response, RRes and JIC stated that 100 N would allow for diversity between the varieties to be identified. KHK asked DEFRA to clarify its objective for this experiment. Is there a possibility of substituting this money into another project area? A screen of *T. monococcum* or the Watkin collection for take all resistance was suggested by TJ. Another suggestion was a study of second wheat syndrome. CC and JF to put a proposal for a second wheat syndrome study together and circulate by the end of July (Action item - completed).

BV clarified that the purpose of the trial was to obtain information on sources of diversity for priority traits, and an insight into the nature of this diversity. The traits addressed would depend on the priorities defined and feasibility. There were concerns the N trial would not be particularly useful/informative.

### **Year two overview of research goals**

#### **Robert Koebner**

RK reported on JIC's research goals for year two. A revised subset of UK accessions that encompass the broadest diversity in the UK will be produced. Up to 200 ESTs/SSRs will be mapped in at least two populations but a single population (Avalon v Cadenza) has currently been agreed upon. Once this has been mapped the

necessity to map a further population will be evaluated. Genotyping of Gediflux has been set at a minimum of 50 SSR loci. With regard to hexaploid mutagenesis, the primary mutagenesis has been performed. Expect ca 5-6000 M<sub>1</sub> plants to be sown by October 04. M<sub>2</sub> seed will be generated by February 05 for planting in March/April 05.

EST-SSRs will be replaced by STMP and standard SSRs

Need to check Avalon x Cadenza cross for variation in priority traits then decide whether to map another cross, eg Spark x Rialto for sprouting.

Hexaploid diversity – will complete genotyping (50 SSR loci) of Gediflux set (500 lines).

Mutagenesis – plant 5-6,000 M<sub>1</sub> plants by October 2004.

Why and how the markers are chosen?

Is it not better to first characterise phenotypes then do the mapping? Since it is OK to set up a reference population and develop marker references based on it, but how to proceed beyond the population entirely depends on the traits.

### **Kim Hammond-Kosack**

KHK reported on the year two goals for RRes Objective 6. Two *T. monococcum* accessions will be selected, seed from a single plant multiplied and a M<sub>2</sub> mutagenised population created. Up to two F<sub>2</sub> mapping populations will be created and phenotyped for their disease response. PCR TILLING will be used to identify variant alleles of SGT1, RAR1 and NPR1 in the diploid accession collection. Specific allele:trait associations will be identified. This will help to create resistance to pathogens by identifying the most affective alleles. The diploid accession population comprises 100 plants. If there is no variation in the three selected genes in these plants, the EMS mutagenised population created from one JIC line will be used.

Questions

If variant alleles are identified, how easy will it be to move these alleles into hexaploid wheat? Is there any information regarding the ease of crossing into hexaploids? Answer - Yes crosses to hexaploid wheat, and introgression into hexaploid wheat are possible in combination with cytogenetics (chromosome counting) .

Do you use single seed descendants for EMS? Answer – No we intend to using 600 M<sub>2</sub> families from a single mutagenised line from the John Innes Centre. This line is called V97031.

Are NPR1, RARa/SGT1 polymorphic in *T. monococcum*? Answer – We will explore this in year 2 of WGIN for each gene.

Are there only three genes affecting resistance? Answer - No, of course not,

but they are some of the key signalling components of innate (activated) resistance in both cereal and non-cereal species. Recently reviewed by Hammond-Kosack and Parker (2003) Deciphering plant-pathogen communication: Fresh perspectives for molecular resistance breeding. *Current Opinions in Biotechnology* 14, 177-193.

Are you planning to confirm the roles of these genes? Answer - Yes, in the first instance by exploring the relationship between specific allele variants and null alleles and the lines susceptibility / resistance to a range of cereal plant pathogens. We will then test this relationship by combining different allele combinations into single lines by sexual crosses and 'within-the-gene' marker selection.

If no gene knock-outs are identified, how can you correlate variant alleles with phenotypes? Answer - See above.

How do these genes work in hexaploid wheat? Answer – It is possible that in hexaploid wheat that only expression from the A, B or D genome is contributing to the trait phenotype. Alternatively, the trait phenotype is the result of the combined and simultaneous expression from two or three genomes. By developing allele specific PCR primers for the A, B and D copies of the gene, it will be possible to examine allele expression patterns in both healthy and pathogen attacked plants.

Do these genes interact with R genes? Answer - There is no published work showing that Rar1, Sgt1 and Npr1 proteins interact with any of the known classes of plant disease resistance proteins.

Can you find specific associations between the variant alleles of these genes and R genes. Answer – In year two of WGIN we shall be looking for these types of associations with particular pathogenic species.

### **Andy Phillips**

AP reported on the year two goals for RRes Objective 9. The M<sub>2</sub> population will be planted, any tall plants identified and leaf material collected for genomic DNA preparation. High throughput TILLING will be established using this material. 8-fold pooling and 96-well formats will be tested. Individuals will be screened for mutations in Rht3 through TILLING. The problem of contaminating aneuploids was addressed. Is it possible that the loss of a chromosome could result in these becoming tall? The aim of this TILLING project is to introduce additional mutations to Rht3. Mutations in the C terminus of Rht3 should knock down the activity of this gene. There is evidence in *Arabidopsis* for suppressor mutations in GAI. It is possible that the chance of seeing a phenotype is low but as Rht3 is a gain of function mutation this chance is greatly increased. Additional TILLING targets have been put forward including GA 20 oxidase. Australian studies indicate a major QTL for PHS in barley is linked to GA20 oxidase enzyme.

Regarding contamination, what is the percentage of tall plants would you expect to see? 5% was the answer.

Can you use SSRs to check whether the plants with the tall phenotype are from contamination or revertants? Yes, assuming that all the contamination is from an identifiable source. Good idea.

Can you choose to work with another gene avoiding the contamination problem? Yes, GAox20, potentially controlling stem height and germination, could be another good candidate.

**BBSRC Crop Science Review (document to be made available)**

Peter Shewry

Key message is that more effort will be put for crop research, balancing BBSRC research focuses on both model and crop plants. Hence, it is good news for crop research.

What is the timetable for this to be implemented? Could take quite some time (years?)

Do you think that it will end up with more wheat research than Arabidopsis research?

Is it possible to join the effort from BBSRC and Defra together?

Perhaps, to attract more people to work with wheat, we need to get interesting traits (biological questions)!

**Forthcoming events**

27<sup>th</sup> October 2004 - next management meeting. To be held at RRes or BB. Venue to be confirmed. (Action item),

A short presentation from N Scoping study will be presented by John Foulkes. Also a presentation on the new Green Grains project, and results from the WGIN year 1 field trial.

Stakeholders meeting – 29<sup>th</sup> November at Rothamsted Research

8<sup>th</sup> -13<sup>th</sup> December – visit to CIMMYT - John Snape is co-ordinating the travel arrangements and agenda for this scientific visit