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SID 4

Annual/Interim Project Report for Period 2010

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Project details

1. Defra Project code: IF0146
2. Project title: Defra - Wheat Genetic Improvement Network - WGIN
3. Defra Project Manager: Dr. Katherine Bainbridge
4. Name and address of contractor: Rothamsted Research, Harpenden, Herts
   Postcode AL5 2JQ
5. Contractor's Project Manager: Prof Kim E Hammond-Kosack
6. Project: start date 01/12//2008
   end date 31/11/2013

Scientific objectives

7. Please list the scientific objectives as set out in the contract. If necessary these can be expressed in an abbreviated form. Indicate where amendments have been agreed with the Defra Project Manager, giving the date of amendment.

01 Project management (RRes); 02 Production of Near Isogenic lines (JIC); 03 The Avalon x Cadenza Mapping population (JIC); 04 Paragon gamma and EMS mutagenised populations (JIC); 05 AE Watkins and Gediflux collections (JIC); 06 New mapping populations to align WGIN 2 with the International wheat genome sequencing (JIC); 07 Insect Resistance (RRes); 08. Nitrogen use efficiency (NUE) and Quality QTLs linked to NUE (RRes); 09 Drought tolerance (University of Nottingham); 10 Take-all disease (RRes); 11 Introgression of extreme resistance to Septoria leaf blotch from Triticum monococcum into hexaploid wheat (RRes);12 Exploring the Interconnections between the three soil based traits; 13 Grain archiving; 14. Sub-contractor projects (To be determined during the project) 15. Website; 16 Electronic Newsletter; 17 Annual Stakeholders Forum; 18 Focussed workshops; 19 International collaborations; 20 Publicity

Summary of Progress

8. Please summarise, in layperson's terms, scientific progress since the last report/start of the project and how this relates to the objectives. Please provide information on actual results where possible rather than merely a description of activities.

Objective 1. Three management meetings were held during the year. (1) 26th February 2010 at Syngenta at Whittlesford, Cambridge, (2) 12th August at JIC, Norwich and (3) 4th November at RRes, Herts. Each was well attended by the funded researchers, other non-funded wheat scientists and the commercial wheat breeders. The progress on the 11 wheat research objectives (2 through 13) is summarised in an accompanying word document.

Objective 14 Subcontractor projects:
Subcontractor project A: Exploring the use of Δ18O and total mineral ash content in wheat as a new tools for phenotyping wheat with respect to water inputs –
John Foulkes, University of Nottingham: Plant dry matter samples from the field experiment in 2009/10 at Nottingham under objective 9.1 have been collected, milled and encapsulated. The specific samples funded with the sub-contractor funds are: (i) A total of 108 flag leaf samples (18 varieties x 2 irrigation treatments x 3 replicates) for 18/O Δ isotope analysis and (ii) 108 flag leaf samples for 13C Δ istope analysis. These samples will be analysed by the isotopes lab in January 2011.
Subcontractor project B: Non-destructive screening of WGIN Paragon mutants for grain NUE traits - Richard Weightman, ADAS: Following transfer of the wheat grain reference samples from SCRI to Aunir in summer 2010, the samples were scanned and new calibrations built for the FOSS 6500 NIR platform. The initial transfer of the samples from SCRI to ADAS was delayed, which meant and this has affected all the subsequent milestones. The development of the new calibrations was completed by the end of August, and ready for transfer to JIC, but it was then postponed as it would have coincided with autumn drilling. The final phase of practical work will now commence at JIC on 10th January when the necessary staff will be in place and the equipment will be installed in the laboratory. It is anticipated that the work will be completed and the final report delivered by 30th April 2011 (original end date 30th November 2010).

Objectives 15, 16 and 17. The WGIN website was kept up-to-date regards meetings reports, the two new newsletters and some datasets from year two. The remainder will be added in January 2011. Newsletters were released in May and November. The stakeholders meeting at RRes was very well attended in Nov with over 100 participants and in particular more participants from the farming community. This event was subsequently reported in the farming press. The stakeholder ppts are available from the website.

Objective 19. In June UK wheat scientists and commercial wheat breeders met for a joint workshop in Novi Sad with members of the East European wheat community. Twelve of the UK scientists participating were funded by a BBSRC ISIS application awarded to John Snape. Results from many parts of WGIN project were presented and discussed. Included was a trip to wheat field trials, which contained the current elite germplasm as well as an extensive wheat diversity collection. Many traits in common were identified.
Objective 20. The scientific outreach activities were numerous, with displays by both JIC and RRes staff on specific WGIN objectives at Cereals 2010, via field demonstrations and talks at the respective sites and engaging the media and the farming press.

Amendments to project

9. Are the current scientific objectives appropriate for the remainder of the project? ..... **YES** ☒ **NO** ☐

If **NO**, explain the reasons for any change giving the financial, staff and time implications.

Contractors cannot alter scientific objectives without the agreement of the Defra Project Manager.

Objective 10 and 11. Introgression of the Take-all and Septoria resistance trait from T. monococcum into Hexaploid wheat (RRes).

In total 28 F1 plants were generated via sexual crossing between the two species, followed by embryo rescue at 13 days post - anthesis. These rooted plants were transferred into soil between Dec 2009 and Feb 2010. Following a 4 week period in vernalisation all the plants were potted up and transferred to a high quality glasshouse along with vernalised hexaploid wheat plants in preparation for the 1st backcross. Unfortunately, the F1 plants failed to produce flowering tillers, or when the ears emerged they lacked anthers containing viable pollen. Only a few died. The 12 non-reproductive plants were placed back in vernalisation for an additional 10 weeks. From this later group, one plant subsequently generated ~200 self seed, from about 6 tillers. The rest of the F1 plants failed to produce reproductive tillers.

The successful cross was between the winter hexaploid Avalon with excellent bread making quality and the T. monococcum accession MDR037. MDR 037 is tall and has both a larger ear size and broader leaves than most of the other T. monococcum accessions in the RRes collection. MDR037 is highly resistant to all isolates of Septoria tritici, but is susceptible to the take-all fungus.

This lack of F1 fertility has seriously delayed this introgression objective. The plan now is to interact with both Professor Ian King at the University of Nottingham and Dr Marta Molnar Lang, the cereal cytogeneticist at the Agricultural Research Institute, Martonvasar, in Hungary to establish a reliable protocol to proceed with the backcrossing either in the glasshouse (UoN) or the field (M). It is possible that embryo rescue will have to be used to achieve a successful backcross to further introgress the resistance to Septoria trait. But we anticipate at least a 1 year delay in achieving this objective 11.
So far we have NOT been able to generate a viable and reproductive F1 between hexaploid wheat and a T. monococcum line containing the take-all resistance trait. Discussions with two wheat introgression experts are now planned for early 2011 to try to revised F1 production protocol. Several protocols will be considered.

Progress in relation to targets

10. (a) List the agreed milestones for the year/period under report as set out in the contract or any agreed contract variation.

It is the responsibility of the contractor to **check fully that all milestones have been met** and to provide a detailed explanation when they have not been achieved.

Milestone		Target date	Milestones met	
Number	Title		In full	On time
01	Project management	30/11/2013		✓
02	Production of Near Isogenic Lines	01/03/2013		✓
03	The Avalon x Cadenza mapping population	30/11/2013		✓
04	Paragon gamma and EMS mutagenised populations	01/01/2011		✓
05	AE Watkins and Gediflux collections	01/07/2011		✓
06	New mapping populations	01/03/2013		✓

07	Insect Resistance	01/04/2010		✓
08	Nitrogen use efficiency (NUE) and Quality QTLs linked to NUE	30/11/2013		✓
09	Water use efficiency and drought tolerance	30/11/2011		✓
10	Take-all disease (introgression part only)	30/11/2013		no
11	Introgression of extreme resistance to Septoria leaf blotch from Triticum monococcum into hexaploid wheat	30/11/2012		no
12	Exploring the Interconnections between the three soil based traits	30/11/2013		✓
13	Grain archiving	30/11/2013		✓
14	Sub-contractor projects	30/11/2013		✓
15	Website	30/11/2013		✓
16	Electronic Newsletter	30/11/2013		✓
17	Annual Stakeholders Forum	30/11/2013		✓
18	Focussed workshops	30/11/2009	✓	
19	International collaborations	30/11/2013		✓
20	Publicity	30/11/2013		✓

(b) Do the remaining milestones look realistic?..... **YES** ☐ **NO** ☐
If you have answered **NO**, please provide an explanation.

Publications and other outputs

11. (a) Please give details of any outputs, e.g. published papers/presentations, meetings attended during this reporting period.

Peer Reviewed:

Barraclough PB, Howarth JR, Jones J, Lopez-Bellido R, Parmar S, Shepherd CE, Hawkesford MJ (2010) Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. Eur J Agron 33, 1-11. [50:10:40]

Parry MAJP, Hawkesford MJ (2010) Food security: increasing yield and improving resource use efficiency. Proc Nutr Soc 69, (online). doi:10.1017/S0029665110003836 [50:50:50]

Gegas V, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, Doonan J, Snape J. A genetic framework for grain size and shape variation in wheat. Plant Cell 22 (4) 1046-1056 2010. Plant Cell. First published online April 2, 2010; 10.1105/tpc.110.074153

McMillan, V. E., Hammond-Kosack, K.E. and Gutteridge, R. J. (2010) Evidence that wheat varieties differ in their ability to build-up inoculum of the take-all fungus, Gaeumannomyces graminis var. tritici, under a first wheat crop. Plant Pathology. Article first published online: 26 October 2010 DOI: 10.1111/j.1365-3059.2010.02375.x. Due out April 2011.

Simon Griffiths, James Simmonds1, Michelle Leverington, Yingkun Wang, Lesley Fish, Liz Sayers, Leodie Alibert, Simon Orford, Luzie Wingen, John Snape. Meta-QTL analysis of the genetic control of crop height in elite European winter wheat germplasm. Accepted by Molecular Breeding DOI 10.1007/s11032-010-9534-x

Thomas Howard, Nur Ardiyana Rejaba, Simon Griffiths, Fiona Leigh, Michelle Leverington-Waite, James Simmonds, Cristobal Uauya, Kay Trafford. Identification of a major QTL controlling the content of B-type starch granules in Aegilops. Accepted by Journal of Experimental Botany

Non-Peer reviewed:

Hawkesford MJ and Howarth JR (2010) Transcriptional profiling approaches for studying nitrogen use efficiency. In 'Nitrogen metabolism in plants in the post-genomic era' eds: C Foyer and H Zhang, Blackwell Publishing, in press.

Parry MAJP and Hawkesford MJ (2010) Genetic approaches to reduce greenhouse gas emissions: increasing carbon capture and decreasing environmental impact. In: Climate Change and Crop Production (ed. M.P. Reynolds) CAB International. Pp139-150. [50:50:50]

Popular press articles:

Oral Scientific Presentations:
February 2010 Kim Hammond-Kosack, University of Nottingham final year undergraduate Plant Sciences students - The WGIN2 project - overview of aims and objectives.
March 2010, Simon Orford: Poster displayed at MONOGRAM workshop: WGIN2 Genetic Resources at the JIC
May 2010, Malcolm Hawkesford: Canada, Calgary, the Nutrient Forum, invited participant
May 2010, Malcolm Hawkesford Canada, Edmonton, invited seminar (NUE Summary)
May 2010, Malcolm Hawkesford:.Las Vegas, iPlant Conference, invited attendee/forum discussion contributor on plant nutrition
June 2010, Malcolm Hawkesford: Cereals 2010, display and poster
June 2010 Kim Hammond-Kosack and Richard Gutteridge, Cereals 2010 take-all display and posters and T. monococcum Septoria display and poster
June 2010, Simon Orford: Genes from the Past Crops for the Future - Diversity and Direction of Watkins demonstrated
June 2010, Malcolm Hawkesford: .Novi Sad – BBSRC ISIS visit, invited participant/speaker (Investigating nitrogen use efficiency in modern wheat germplasm)
July 2010, Malcolm Hawkesford : Japan – Nitrogen 2010 talk (Nitrogen use efficiency in wheat: traits and quality issues)
October 2010. Three posters describing the take-all disease problem for wheat and the new lowTAB trait were presented at the KTN conference held in Edinburgh.
November 2010, Malcolm Hawkesford: Danish Biotech forum – invited opening plenary speaker

(What is nutrient use efficiency, and can it be manipulated?)
December, 2010, Malcolm Hawkesford: .Invited named (AEV Richardson) seminar (Dissecting nitrogen use efficiency in modern wheat, or who cares about nitrogen, and what can we do about it?) at Waite Institute, Australia,
December, 2010, Malcolm Hawkesford: AAB NUE/WUE Conference, Grantham, Lincs, – co-organiser (December)

Scientific Discussions:

Scientific Outreach activities / articles

January 2010, Simon Orford: Land-skills East Leadership Course Easton College, Presentation to Industry leaders regarding the role of WGIN2
March 2010, Simon Orford: Visit to 50 A level students to present the role of WGIN2 in UK Wheat Breeding
April 2010, Malcolm Hawkesford: BBC Countryfile item on food security described and was filmed on WGIN plots.
May 2010 Richard Gutteridge, Rothamsted Research open week-end, Trailer tours of the farm which included the WGIN diversity field trials Over 7000 visitoirs attended the site and ~ 1000 took the farm tour.
June 2010, Simon Orford: FOJIC event: Genes from the Past Crops for the Future - Diversity and Direction of Watkins demonstration to 90 members of the public including newly elected local MP
June 2010 Richard Gutteridge Demonstration of the WGIN take-all trials, Watkins/Gediflux, and T. monococcum to a group of 20 visiting Australian and New Zealand agronomists
June 2010 Richard Gutteridge Presentation of the WGIN take-all date on the low inoculums build up trait and the protocols used to Recommended List wheat committee.
June 2010 Articles in the farming press on the WGIN take-all project, Farmers Weekly and Farmers Guardian on line. Following interviews with Richard Gutteridge and Kim Hammond-Kosack at Cereals.
John Foulkes A poster on the work under WGIN Activity 9 Drought Tolerance was presented at two University of Nottingham Open days during 2010.
Summer 2010 BBSRC business - The WGIN T. monococcum trial and Kim Hammond-Kosack talking to farmers attending Cereals 2010 featured in the out and about section of this magazine.
October 2010 Kim Hammond-Kosack. Letters and follow up discussions with the local Redbourn Parish Council and St. Albans District Council, Herts on the importance of wheat production in SE of England following proposals to build significant housing developments on green belt and non-green belt agricultural land. Several planning applications on green belt sites subsequently abandoned and alternative brown field sites within the urban space are now under consideration.
October 2010 Richard Gutteridge Presentation on novel approaches to Take-all control to Bayer CropScience.

(b) Have opportunities for exploiting Intellectual Property arising out of this work been identified? ..... **YES** ☐ **NO** ☒

If **YES**, please give details.

This project is entirely IP free.

(c) Has any other action been taken to initiate Knowledge Transfer?..... **YES** ☒ **NO** ☐

If **YES**, please give details.

An application was successfully made to the Technology Strategy Board (TSB) initiative called 'New Approaches to Crop Protection'. In this new project, the novel trait identified with WGIN called LOW take-all inoculum build up (LowTAB) will be advanced into the current elite UK commercial wheats and tested This project started in October 2010 and is funded for 5 years. The funded partners are Rothamsted, and the wheat breeders RAGT (lead Dr Sarah Holdgate), KWS and Limagrain. In putting the TSB application together staff at the KTN were regularly consulted throughout the process. This project success was recently publicised in a KTN leaflet and at a KTN workshop held in Edinburgh in October 2010

Future work

12. Please comment briefly on any new scientific opportunities which may arise from the project.

The effect of combining existing fungicides against the take-all fungus in a 1st wheat situation in combination with the lowTAB trait is being explored in 2010 and 2011 with the appropriate commercial companies.

Declaration

13. I declare that the information I have given is correct to the best of my knowledge and belief.

Name	Professor Kim Hammond-Kosack	Date	26th January 2011
Position held	The overall PL on the WGIN 2 project		

Summary of Progress on the eleven Science Objectives:

Objective 2 Production of Near Isogenic Lines NIL (JIC);

Avalon x Cadenza NILs:
Using MAS (Marker Assisted Selection) Near Isogenic Line (NIL) development has successfully multiplied (Backcross2Filial2) BC2F2 height and flowering time targets. This material has entered field trials at JIC for harvesting in 2011
Material for 1B heading / late, 1D heading early, 2A height / short, 2D height / tall, 2D height yield, 3B height / short, 5A yield, 6A height, 6B height, 7B and 7D yield (all to Avalon). 1B heading early, 1D heading, 2D height, 3B yield, 2A height / tall, 2D height, 6A height, 6B height and heading, 3B height (all to Cadenza) are at the BC2F2 stage

Rialto x Savannah NIL:
Completed BC1 production for 3D stay green trial into Rialto, Savannah and Paragon

Lr19 NILs:
Leaf rust resistance gene Lr19 from Agropyron elongatum is being followed in 2 sets of crosses, Oasis, Kamb1 and Wheatear x Paragon and Oasis, Kamb1 and Wheatear x Alchemy. Analysis using the Lr19 specific marker Complete to backcross3.

Malacca x Hereward NILs:
A range of bread-making quality traits and measurements including loaf volume and cell number are being followed in this cross. Completed third backcross using MAS.

Objective 3 The Avalon x Cadenza Mapping population (JIC);

A new version of the Avalon x Cadenza map has been released for use on the WGIN website. The JIC map has been created for general use as a pre-made tool using "Joinmap". It comprises 28 linkage groups, covering 2000cMs. A mixture of marker types (SSRs, DaRT, COS, AFLP and perfect markers) has been incorporated to give the best overall coverage, and co-segregating markers have been kept to a minimum. This map gives a good overview of the cross, and would be suitable for QTL analysis. Quality control on this map has been high, with all data checked, and the map order of the markers verified using a new web based mapping tool called Threadmapper (http://cbr.jic.ac.uk/threadmapper). All mapping data is available so that it is possible to create your own map. This may be useful for cross referencing purposes. See objective 10 where this new A x C map has already been used to map new traits.

Objective 4 Paragon gamma and EMS mutangenised populations (JIC);

Gamma:
450 M3 lines (250Gy) of Paragon developed with DNA extracted. A further 1000 M3 and 3500 M2 lines of Paragon developed (all 250Gy).

EMS:

EMS: 6500 M6 lines developed from field trials. Populations developed from these mutants (heading late, height short, stay green early / late) to F3 with spring cultivars and sown to field trials harvested 2010. Stay green candidate lines sown in Autumn 2010 for identification of the stay green phenotype within heterozygotes families.

Objective 5 AE Watkins and Gediflux collections (JIC);

AE Watkins:
The AE Watkins collection has undergone extensive genotyping with forty informative SSR and three COS markers screened across the entire 1060 line collection. This has enabled a valuable assessment of the allelic content and diversity of the collection.
Gediflux:
The Gediflux collection has been bulked under glass to enable sufficient stocks for field sowing in autumn 2010. An assessment of the comparative genotypic diversity between these two populations has been made through SSR data.

Objective 6 New mapping populations (JIC)

See objective 9.3 below where additional mapping populations are underway specifically for the needs of the Water Use Efficiency joint project between UoN and JIC.

Objective 7 Insect resistance in wheat: Cereal aphids (RRes) (note - this trait was only funded to be studied in year 1)

Objective 1. To explore whether the differential response of hexaploid wheats to two different cereal aphid species has a genetic basis.

Milestone 1. Determine the differential susceptibility to two cereal aphid species of targeted lines from the Spark x Rialto mapping population.

In year 1, the preference indices were determined for UK cereal aphids *Rhopalosiphum padi* and *Sitobion avenae* against seventeen selected lines from the Spark x Rialto mapping population plus the parental lines. As seen in trials with other aphid species, the most and least preferred lines were not the same for *R. padi* and *S. avenae*. However, there were a few lines where the responses coincided and one in particular, SR120 that was very susceptible to both aphid species. The Mean Relative Growth Rate (MRGR) was determined for each aphid species on eight of the lines, chosen from the most and least preferred, plus the two parental lines. Replicated groups of neonate nymphs were weighed in batches of five on a microbalance and then transferred to clip cages on the first leaf of 7-8 day old seedlings of Solstice, as the standard variety, or of the test line. The seedlings were kept in a controlled temperature room (20 +/- 2°C, 16:8 h L:D, 40% RH). Surviving nymphs were re-weighed in their batches after 7 days and the MRGR was calculated:

MRGR = (ln 7day weight – ln birth weight) / number of days

All data were subjected to ANOVA and there were no significant differences in MRGR compared to Solstice for either aphid except for *R. padi* on its least preferred line SR67. With the exception of SR67, settlement preference of alatae did not correlate with development rate of nymphs. Since SR120 was most preferred, and Spark preferred to Rialto by both species, we looked at leaf morphology of all three lines

and Solstice under UV with a high powered stereo microscope. Spark had very few leaf hairs on both abaxial and adaxial leaf surfaces in contrast to Rialto and Solstice, which both had lots of leaf hairs on both surfaces. However, SR120 also had extensive leaf hairs on both surfaces, which ruled out that particular aspect of leaf morphology from affecting aphid settlement in this case. These data are now being checked to determine whether there is any genetic basis to the effects with the possibility of creating a new mapping population between SR120 and SR67. The approach and key findings from this one year study were reported at the Nov Stakeholders meeting within the project overview ppt given by KHK.

A final report on this study will be presented at the February 2011 management meeting and this will be accompanied by a final report. Both will then be placed on the WGIN website.

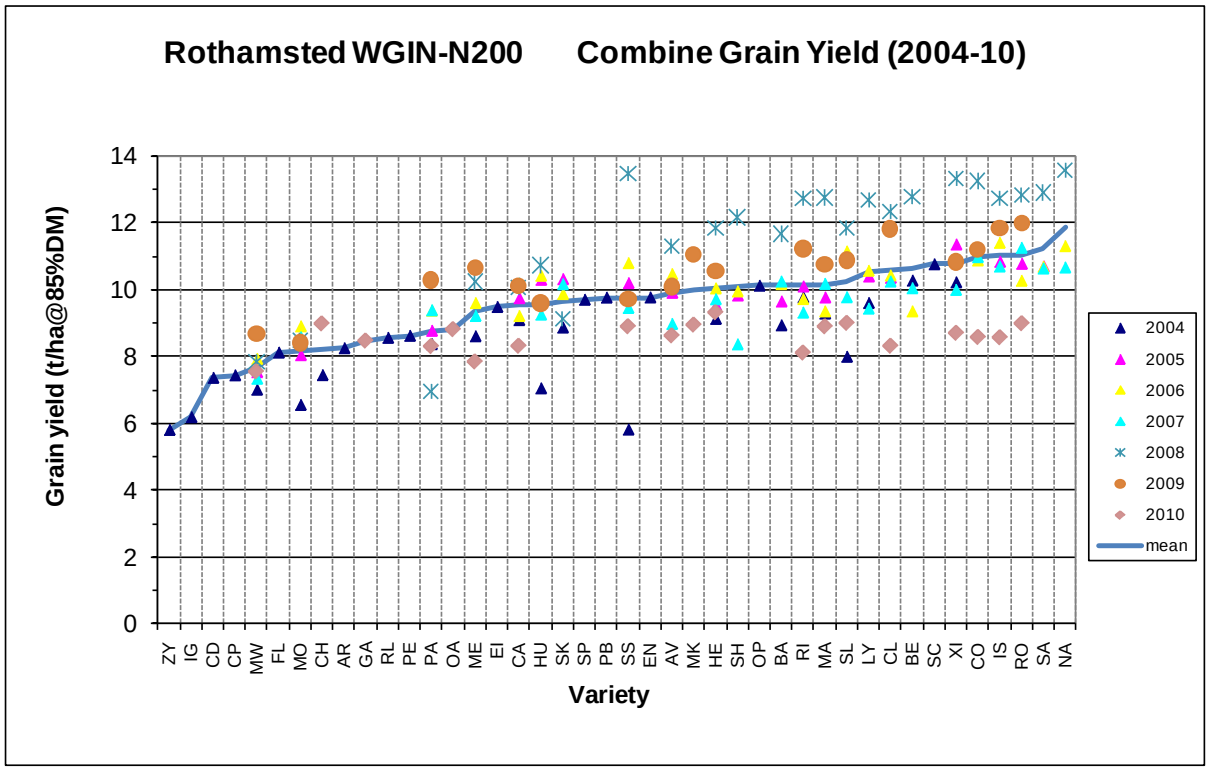
Objective 8. Nitrogen use efficiency (NUE) and Quality QTLs linked to NUE (RRes)

Two field trials were planted in the autumn of 2009 and harvested in August/September 2010. In both cases grain and straw yields were recorded and samples taken for nitrogen analysis (in progress). In addition canopy spectral reflectance data were collected to assess post anthesis canopy longevity. These trials were made available to the community for phenotyping/sub sampling. The first of these trials (The Diversity trial) examined 25 varieties subject to 4 levels of N fertiliser application (0, 100, 200 and 350 kg/ha) in a triplicate design. The varieties consisted of 19 elite current or older varieties (see figure showing yield response) and 6 lines selected double haploid lines from the mapping population (Avalon x Cadenza). A summary of performance of the commercial varieties during the first and second WGIN projects is shown in the figure (see below). The selected double haploid lines (4 lines overlapping with 2009, chosen for contrasting NUtE, leaf N export patterns and rates of senescence) were subject to a more detailed yield component analysis following anthesis and at final harvest. The second trial (The Avalon x Cadenza Mapping Population Trial) consisted of all 204 lines of the mapping population, grown in triplicate at 200 kg N/ha. This trial has now been completed 3 times at low N and 2 times at high N. Combining these data will enable identification of nitrogen responsive nitrogen uptake ability and utilisation efficiency QTLs. In addition the trials were phenotyped and sub sampled for a number of separately funded projects including assessments of grain protein quality, transcriptome, canopy structure and senescence, and nitrogen content at and just after anthesis.

The phenotyping data (yield, NUE parameters, canopy senescence) being collected on the mapping population is being averaged over multiple years/sites and for two N inputs, prior to final mapping and definition of QTLs.

In the autumn of 2010, the Diversity trial was planted with 25 varieties. Two of the double haploid lines were removed (the high/low NUtE lines) and 2 new commercial elite varieties were added (Stigg and Crusoe). These two varieties represent a source of material which has been derived from the use of Triticum dicoccoides. With high levels of disease resistance - particularly to *Septoria tritici* these represent novel germplasm. Both have a 'stay green effect'. Crusoe includes a dicoccoides segment associated with non-glaucousness, stay green and higher yield in UK environments (Simmonds et al 2008, Euphytica). This segment is being dissected in detail at JIC.

Stigg is a candidate for the 2011 Recommended List and Crusoe has been promoted to Recommended List Trials for 2011.



Objective 9: Drought Tolerance (UoN):

Objective 9.1 "To identify physiological traits for WUE"

One replicated (3 reps) field experiment has been successfully completed in 2009/10 examining physiological traits, water-use efficiency (WUE) and grain yield of 18 varieties under irrigated and unirrigated conditions at Nottingham University. The following measurements were taken in all sub-plots: (i) combine grain yield and yield components, (ii) biomass, shoot number, green area and DM partitioning at GS31, GS61 and harvest, (iii) % stem water soluble carbohydrate at GS61+10d, (iv) leaf senescence kinetics for flag-leaf, L2 and L3 at 3-4 day intervals post-anthesis, (v) water-use efficiency estimated by $^{13/12}C$ isotope discrimination (Δ) of flag leaf samples at GS61 and grain DM samples at harvest and (vi) leaf transpiration estimated from $^{18/16}O$ isotope discrimination (Δ) of flag leaf samples at GS61. Additional measurements were carried out for a sub-set of eight varieties in the unirrigated treatment only: (i) stomatal conductance/photosynthetic rate (Licor 4600 gas exchange analyzer) and (ii) water uptake from gravimetric analysis of soil cores (indicative of root activity).

Grain yield overall was reduced from 10.34 to 8.48 t ha^{-1} ($P< 0.001$), with the range in yield losses varying amongst varieties from 11% (Soissons to 22% Xi19). Data analysis is currently ongoing for senescence kinetics, canopy temperature and Licor gas exchange data. Preliminary analysis indicated a highly significant correlation between canopy temperature and grain yield under both irrigated and unirrigated conditions. Leaf and grain samples for ^{13}C Δ and ^{18}O Δ isotope discrimination analysis have been milled and encapsulated prior to isotope analysis in January 2011. A field experiment with the same experimental design has been successfully established at University of Nottingham in October 2010. The experimental measurements as outlined for 2009-10 above will be carried in this experiment.

Objective 9.2 "To identify robust QTLs for water-use efficiency"
One DH mapping population (Rialto x Savannah) will be phenotyped at two sites in 20010/11 and data used for QTL analysis using an existing molecular marker map at JIC for this population, augmented with additional SSR markers as required. 94 DH lines and the two parents were sown in October 2010 in a replicated field experiment at each of Nottingham University (2 reps) under irrigated (trickle irrigation) and unirrigated conditions and at JIC (3 reps) under unirrigated conditions.
In all sub-plots in both experiments, anthesis date, plant height, combine grain yield and yield components will be measured. In the Nottingham experiment additional measurements will be carried out: (i) % stem water soluble carbohydrate at GS61+10d, (ii) leaf senescence kinetics for flag-leaf, L2 and L3 at 3-4 day intervals post-anthesis and (iii) water-use efficiency estimated by ^{13}C (Δ) of grain DM samples at harvest.

Objective 9.3 "To develop one DH population for drought-tolerance research".
Based on available information from previous publicly funded projects seven parental wheat lines contrasting for high/low WUE (according to^{13}C Δ) were crossed in May 2010 at JIC to produce all possible combinations of F1 seed. Seed from all F1 crosses has been sown in the glasshouse at JIC in October 2010. One F1 cross will be selected in January 2011 in consultation with the UK breeders and crossed with maize using the maize pollination technique at JIC to develop (after embryo rescue and chromosome doubling with colchicine during 2011) one DH population with 100+ lines as a resource for future UK drought tolerance research studies.

Objective 9.4, "To identify novel alleles for the AE Watkins and Gediflux collections"
A visual assessment of senescence (% flag leaf green area) was carried out on the AE Watkins lines in a field experiment at JIC in July 2010 during late grain filling. Further visual assessments of senescence during grain filling and flag leaf rolling will be carried out in the field both at JIC and Nottingham on the AE Watkins lines in 2011.

Objective 10. Resistance to take-all disease (RRes)

10.1 and 10.2 To screen the AE Watkins Collection and an 'improved' Gediflux Collection to identify potential sources of resistance to the take-all fungus under field conditions.

The A.E.Watkins wheat collection dates back to the 1930's and approximately 800 lines have been rejuvenated at JIC. This unique collection may contain alleles and allelic combinations for disease resistance that breeders may have left behind. To explore this possibility, a series of field experiments, started in the autumn of 2007, were designed to test the lines available under natural disease pressure. As seed was in short supply, only 0.5m^2 plots (containing 3 rows) was possible and these were incorporated into an alpha designed experiment (see 2009 report). Only nitrogen, growth regulator and herbicide were used in 2008 and in 2009 and 2010 an eyespot treatment was included. All diseases were in significant amounts in 2008. Some lines showed Septoria resistance above that of the controls but we have not been able to confirm this finding as in both 2009 and 2010 all leaf diseases have only

been very slight across the whole experiment. These lines have been again included in the 2011 experiment. Stem base diseases were only assessed in 2008 and 45 of the 740 lines showed high resistance to eyespot. These lines, together with 33 highly susceptible lines, were further assessed at JIC for the presence or absence of *Pch1*, *Pch2* and the 5A gene, using DNA markers that are known to be genetically linked to the resistances. Initial findings showed that 38 of the 45 resistant lines have no known resistance genes suggesting there may be another novel source of resistance operating.

Take-all assessments have been completed on the 2009 experiment and samples taken in July from the 2010 trial. Combining the results from the first two years a total of 319 Watkins lines were shown to be fully susceptible to take-all and were discarded from the 2011 experiment. Similarly, 24 of the 60 Gediflux lines have been discarded. The 2011 experiment consists of 540 Watkins/Gediflux lines plus 60 plots of controls. This includes the 83 new Watkins lines received from JIC in 2009 for further testing. Root assessments should be completed on the 2010 samples by early February 2011.

10.6 Generation and screening of mapping populations

Two of the *T. monococcum* populations have been taken to the F4 generation and seed will be available in Feb 2011 available for screening for ~85 lines per population. The F4 and not the F6 generation has been selected for initial take-all screening. In total, by combining the results of the field and pot tests 6 Tm accessions have been identified which show the highest level of resistance to take-all. A detailed reciprocal crossing programme is still in progress with these lines to a number of key susceptible genotypes. For the most important crosses F1 seed is already available and F1 plants are now growing to develop F2 populations, but March 2011. DArT marker analysis is partially complete and the subsequent PCA has reveals some of the resistant accessions are genetically quite close to fully susceptible accessions whilst the others resistant accessions are genetically unrelated. Another batch of 16 DNA samples has been sent to Triticarte in Australia to complete the DArT marker analysis for the additional resistant lines of interest. Follow up PCA are planned and these will be combined with the phenotyping and genotyping data arising from the F4 screening in pots. .

10.7 Introgression of non-hexaploid and *T. monococcum* sources of resistance into hexaploid wheat

By Dec 2009 all *T. aestivum* x *T. monococcum* crosses were completed using Avalon and Cadenza with embryo taking place at 14 days post pollination. In total 28 F1 rooted plants were transferred to the glasshouse after 4 weeks in vernalisation. **See below objective 1**1 for an explanation of what happened subsequently (a joint objective). So far we have been able to generate a viable and reproductive F1 between hexaploid wheat and a *T. monococcum* line containing the take-all resistance trait. Discussions with two wheat introgression experts are now planned for early 2011 to try to revised F1 production protocol. Several protocols previously considered are now worth exploring.

10.8 Identification and characterisation of hexaploid wheat germplasm which reduce take-all inoculum build up (TAB) in the soil.

The diversity trial which is designed to look at the nitrogen use efficiency of different bread wheats (see objective 08) contains 24 different wheat cultivars grown as a first cereal crop. Results from these trials showed that wheat cultivars do differ in their ability to build-up the take-all fungus in the soil. A publication on these findings has been prepared and accepted for publication (McMillan *et al*. (2011) Plant Pathology, in press).

At present we only have information on cultivars grown at one nitrogen rate. However, in the future nitrogen application may become more restrictive and information on the inoculum/nitrogen/cultivar inter-action is required. Therefore, in 2010 six cultivars were selected from the 24 which covered the range of low to high builders of inoculum and soil cores taken from all nitrogen rates and replicates. Results from this trial are currently being analysed.

10.9 Explore the genetic basis of take-all inoculum build up using the Avalon x Cadenza mapping population.

In 2009, data from one of the Avalon x Cadenza mapping population trials revealed two possible major quantitative trait loci (QTLs) controlling the **t**ake-**a**ll inoculum **b**uild-up (TAB) trait. This result led to a successful TBS application on the lowTAB trait with three of the UK based wheat breeding companies. This project started in October 2010 for 5 years and will involve additional trials at both RRes and three other sites.

In 2009, soil cores were taken from all 204 A x C line plots to assess their TAB potential and to gain further information on these QTLs. The year was conducive for take-all inoculum build-up, as indicated from other sites at Rothamsted, but unfortunately take-all failed to develop on the site where the 204 A x C lines were grown. The roots of the bioassay plants showed symptoms of *Phialophora* spp. and previous work, at Rothamsted, has shown that the presence of these fungi can delay the development of the take-all disease which may explain the lack of inoculum build-up. This year we have again taken soil cores from all 624 plots in the A x C experiment for TAB to further study this trait. These samples will be assayed in the pot tests over the winter 2011 and spring 2011.

Objective 11. Introgression of extreme resistance to Septoria leaf blotch from *Triticum monococcum* into hexaploid wheat (RRes)

In total 28 F1 plants were generated via sexual crossing between the two species, followed by embryo rescue at 13 days post - anthesis. These rooted plants were

transferred into soil between Dec 2009 and Feb 2010. Following a 4 week period in vernalisation all the plants were potted up and transferred to a high quality glasshouse along with vernalised hexaploid wheat plants in preparation for the 1st backcross. Unfortunately, the F1 plants failed to produce flowering tillers, or when the ears emerged they lacked anthers containing viable pollen. Only a few died. The 12 non-reproductive plants were placed back in vernalisation for an additional 10 weeks. From this later group, one plant subsequently generated ~200 self seed, from about 6 tillers. The rest of the F1 plants failed to produce reproductive tillers.

The successful cross was between the winter hexaploid Avalon with excellent bread making quality and the *T. monococcum* accession MDR037. MDR 037 is tall and has both a larger ear size and broader leaves than most of the other *T. monococcum* accessions in the RRes collection. MDR037 is highly resistant to all isolates of Septoria tritici, but is susceptible to the take-all fungus.

This lack of F1 fertility has seriously delayed this introgression objective. The plan now is to interact with both Professor Ian King at the University of Nottingham and Dr Marta Molnar Lang, the cereal cytogeneticist at the Agricultural Research Institute, Martonvasar, in Hungary to establish a reliable protocol to proceed with the backcrossing either in the glasshouse (UoN) or the field (M). It is possible that embryo rescue will have to be used to achieve a successful backcross to further introgress the resistance to Septoria trait. But we anticipate at least a 1 year delay in achieving this objective 11. Discussions with two wheat introgression experts are now planned for early 2011 to try to revised F1 production protocol. Several protocols will be considered.

The fine mapping of the *TmStb1* locus conferring extreme resistance to Septoria tritici has continued by starting to screen an addition 120 F2 lines (12 plants per line) in one additional population. The seedling assay done in the greenhouse is being used.

Objective 12. Interconnections between the three soil-based explored traits (RRes and UoN)

This final research object will not formally start until project year 2.5, but we have ensured that in the year 1 and year 2 NUE , WUE and take-all trials that there are common genotypes for cross referencing purposes in preparation for the combined data analyses.