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PROJECT TITLE :
Defra – WHEAT GENETIC IMPROVEMENT NETWORK - WGIN

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Project partners: ROTHAMSTED RESEARCH and JOHN INNES CENTRE
Sub-contractors: ADAS and NIAB

SECTION 1. LIST OF THE OVERALL SCIENTIFIC OBJECTIVES

SECTION 2
The details for each of the main objectives

Objective 01 Project management
1A Objectives At least three management meetings took place each year and these well attended by the wheat breeding community and by those representing the nominated UK establishments. However, the BBSRC nominated representative rarely attended these meetings.
1B. Methods & Results The venue for the meetings was always rotated and included RRes, JIC, NIAB, University of Nottingham and ADAS Boxworth
1C. Discussion of results The latest findings from the various research projects were presented and discussed. All accompanyingpowerpoints presentations and the key question / answers / discussion points are to be found in the approved meeting minutes. Both are available on the WGIN website.
1D. Implications An active and engaged network of UK based wheat scientists and wheat breeders was maintained throughout the project.

1E. Future Work Most of the activities and proposed traits for inclusion in the WGIN renewal project where discussed over the last two years of management meetings. Many PhD projects and newly funded projected have arisen as a direct result of WGIN 1. Those up to May 2008 are summarised in this WGIN Newsletter and two others have since started.
1F. Knowledge transfer A far larger number of KT activities were done within the WGIN project that had been envisaged at the outset. For example, living demonstrations plots on the WGIN research and aims were presented at Cereal 2006, 2007 and 2008 by both JIC and RRes. There were visits by members of the Rothamsted Research Association and Friends of John Innes to field trial demonstrations and WGIN talks. Also the management meetings frequently combined with a larger discussion workshop involving large sections of the UK wheat research community. At the management meetings there were also presentations given by invited international wheat community and UK industry, for example, on the DArT technology available in Australia and new technologies available at IDna Genetics Ltd based at JIC.

Objective 2 Plant genetic resources **(JIC)** To characterise at the phenotypic and molecular levels, and make available, the national wheat varietal and specialist genetic lines collection held at JIC
2A Objectives Precise genetic stocks have been characterised at JIC both cytologically and with the aid of molecular markers. Passport information for the materials available can be retrieved from public databases accessible via the JIC website www.jic.ac.uk. This includes the maintenance and characterisation of existing material, as was originally proposed, and new materials produced by WGIN such as gamma induced deletion lines. A substantial commitment of staff time, field, and glasshouse budget has been required to service the high level of demand for germplasm maintained and developed under WGIN.
2B. Methods & Results Lines were characterised at the molecular level using simple sequence repeat (SSR), Diversity Arrays Technology (DArT) and allele specific genic markers (eg Ppd, Vrn, Rht). Cytogenetic analysis was conducted by classical Feulgen staining techniques. Phenotypic assessment was carried out in field and glasshouse.
2C. Discussion of results The results obtained using molecular markers and cytogenetic screens are well established and robust. The materials produced are being used extensively by national and international colleagues, validating the work carried out under WGIN.
2D. Implications Precise genetic stocks are an increasingly valuable resource. The International Wheat Sequencing Programme is based on individually sorted wheat chromosomes that can only be recovered from Chinese Spring aneuploids. The maintenance of this material and associated skills has been strongly supported by this programme.
2E. Future Work The continued support of these resources will allow a large number of follow on projects including physical mapping of the wheat genome, dissection of QTL by deletion mapping, and the introgression of traits identified in exotic germplasm into UK elite winter wheat.
2F. Knowledge transfer The work has provided a key resource for understanding the complex genetics of hexaploid wheat and fed into numerous programmes and projects for the genetic improvement of wheat. Tacit

knowledge of germplasm handling and maintenance has been passed on to resource users.

Objective 03 Genetic mapping and marker development **(JIC)** To populate current maps with genic markers based on EST sequences, and to link the genetic map to a physical one based on BACs. To drive discovery towards informative, function-related and high throughput 'genotyping- compatible' markers.
3A Objectives These objectives have been fully met and exceeded. Work carried out in the WGIN project has led to the delivery of over 1000 new gene based markers to the wheat community. These markers have been mainly mapped in Avalon x Cadenza and Opata x Sythetic.
3B. Methods & Results These markers were based on PCR amplification of the introns as well as 3' and 5' untranslated regions (UTRs) of wheat genes. These genes were annoated in relation to the model genomes of rice and Brachypodium and the physical map of wheat via *Aegilops cylindrica* induced deletion lines.
3C. Discussion of results The Results obtained are robust. Evidence of the utility of this marker set can be seen from it's wide use amongst academic and industrial partners.
3D. Implications The wheat genetic maps of the academic community and industrial community have been brought into alignment with the sequenced genomes of grass relatives bringing the predictive power of gene order from model species to bear on wheat, the major crop of Western Europe.
2E. Future Work The COS marker set is and will continue to be widely used. Future marker development work will convert the platform from a 'one by one' to a massively parallel approach based on next generation sequencing. This work will be reported at future WGIN meetings.
2F. Knowledge transfer The marker set facilitates knowledge transfer between crops because common markers allow the alignment of common traits. For example the company KWS have mapped these markers in Rye and IBERS in oat. The marker platform has been used in a number of studies to map candidate genes. The Defra LINK Hagberg Falling Number Project is a good example of this.

Objective 04. Hexaploid diversity screen **(JIC)** To establish an open-ended (initially largely genotypically-based) database of wheat germplasm relevant to the UK gene pool, for the monitoring of temporal and geographical trends in diversity, and as a resource for future association studies

4A Objectives These objectives were met by the work carried out on the AE Watkins (pre 1930s land races) and Gediflux (Western European high impact winter wheat varieties from 1940) collections.
4B. Methods & Results Field and glasshouse phenotype data was collected for both collections and SSR genotyping carried out for Gediflux. The AE Watkins collection was assessed for functional polymorphisms in major gene effects such as the vernalisation gene *Vrn*.
4C. Discussion of results Phenotypic results were gathered from replicated trials. Genotyping was carried using robust platforms and cross checked with

phenotypic data. For example AE Watkins lines genotyped as spring types using *Vrn* markers were already scored as spring growth habit from trial data.
4D. Implications The results obtained demonstrated the very broad phenotypic and genotypic diversity of these collections, and the presence of new alleles unknown to modern agriculture.
2E. Future Work This work is a springboard for the exploitation of the novel allelic variation present in these collections. A large number of projects outside of WGIN have used this information to include sub sets of this material.
2F. Knowledge transfer The value of the hexaploid diversity screen has been demonstrated in field walks at JIC and RRes attended by WGIN stakeholders, school groups, Friends of John Innes, and industrial partners.

Objective 05 Trait identification **(RRes (1) and JIC(2))** To generate quantitative information on important traits for sustainable agriculture and to provide this knowledge in an accessible database.
5A. Objectives-1 (RRes) Field trials were conducted on a selection of hexaploid wheat cultivars (*Triticum aestivum* L.) and in a nominated mapping population (Avalon x Cadenza) to measure the agronomically important trait 'nitrogen efficiency'.
5B. Methods & Results-1 Crop N-efficiency has two components, N-uptake efficiency (NupE) and N-utilisation efficiency (NutE). NupE is defined as total N-uptake in the above-ground plant material at grain maturity divided by the supply of N during the growing season from soil and fertiliser (NupE = uptake/supply as kg-N/ha/kg-N/ha). NutE is defined as grain dry matter yield divided by total N-uptake by the above-ground plant material at grain maturity (NutE = yield/uptake as kg-DM/ha/kg-N/ha). The trials were conducted at Rothamsted over 5 seasons (2004-05-06-07-08). In all, 39 varieties were assessed at 5 N-rates over 5 years. Not all varieties were grown at all N-rates in all years, but for 24 cultivars we have at least 3 field years of data The varieties were a mixture of old and new UK and continental cultivars including bread and feed wheat. The wheat was autumn-sown after oats in 3 m x 10-20 m plots arranged in randomised blocks. In addition the Avalon x Cadenza population was grown in two years (one at high N and two trials in a second year, both at low N input). Harvesting was in August. Grain and straw dry matter yields and %N (by Dumas' combustion method) were measured and used to calculate total N-uptake, grain-NutE, and grain and nitrogen harvest indices (GHI and NHI). Soil mineral-N to 90 cm depth was measured in February each year. Results were analysed by ANOVA-REML, correlation analysis and varieties were ranked according to performance.

5C. Discussion of results -1 The factor having the biggest effect on yields and N-efficiency parameters was N-rate with variety generally having the smallest effect. There were significant varietal differences however in grain yield, grain %N, total N-uptake and grain-NutE. All 2-way and 3-way interactions (variety x N-rate x year) were significant. This means, for example, that good performance at low N was not always matched by good performance at high N. NutE depended on N-rate, was not correlated with N-uptake, was inversely correlated with grain %N and was correlated with grain yield. Nabim Group 3 and 4 varieties (the ‘biscuit’ and 'feed' wheats) , had the best all-round

rankings with respect to grain yield, N-uptake and N-utilization but were some of the poorest performers with respect to grain-N.

5D. Implications-1 It seems that breeders have inadvertently selected for high NutE in their quest for high yields. There is scope to improve NutE, whilst maintaining grain quality, by manipulating NHI (improving remobilisation of N from straw to grain). NupE might also be improved by manipulating N acquisition by roots

.**5E. Future Work-1** This is required to identify the underlying mechanisms responsible for the observed variation (in all traits). This will involve work on uptake efficiency (probable root trait) and on conversion into yield (canopy traits). Work on the latter is in progress in assessing how post anthesis canopy senescence processes impact on grain yield and nitrogen (Lawes Trust PhD student appointed Oct 2008). Work is also required on identifying varieties and underlying mechanisms which break the inverse relationship between grain yield and nitrogen content (BBSRC application successful 2009). Work on root traits and uptake will form a part of the planned WGIN renewal. In the present project preliminary investigation of the Avalon x Cadenza mapping population has instigated the identification of relevant QTLs. Further work is required to verify these and determine their interactions with N inputs (also planned WGIN renewal).

5F. Knowledge transfer-1 This work has been presented at WGIN management meetings and stakeholder meetings and at national 'Cereals' events in 2007 and 2008. All results are stored in a database at Rothamsted and selective results have been posted on the WGIN website. Papers are in preparation for refereed journals.

5A. Objectives-2 (JIC) Quantitative and qualitative trait data has been collected for the Avalon x Cadenza doubled haploid (DH) population for key sustainability and basic agronomic traits. An essential complement to the phenotypic data collection for the Avalon x Cadenza population was the generation of genotypic information for all 204 individuals. A genetic map was produced for Avalon x Cadenza and used in conjunction with phenotype scores to conduct quantitative trait locus (QTL) analysis. In addition extensive phenotypic data collection has been carried out as described elsewhere for EMS mutant collection, the AE Watkins Collection, and the Gediflux Collection. All genotypic and phenotypic data is available on the WGIN website.

5B. Methods & Results-2 The majority of data has been collected in replicated field trials from 5mx1m plots. Traits measured include height, heading date, plot yield, grain weight (thousand grain weight) spikelet number, grain protein content, grain texture, grain width, grain length, leaf wax, and growth habit. Statistical analysis of the data including genetic map construction and QTL analysis were carried out by trained staff using community standard software (Genstat, Joinmap, and QTL Cartographer).

Data has been collected according to standard operating procedures under the guidance of JICs quality assurance programme. Reliability of data collection and assessment of trait heritability was provided by replication within experiments and across sites and years.

5C. Discussion of results -2 This data set will be invaluable for new as well as existing research projects.
5D. Implications-2 The data has provided the first comprehensive QTL data set for modern UK adapted elite wheat germplasm. It provides a focal point for studies based on this and other resources where genetic maps, QTL, and sequenced genomes can be integrated.
5E. Future Work-1 WGIN trait identification data has provided a unique opportunity for integration of community data. For example the QTL identified are being collated with previously described effects in a QTL meta-analysis. Germplasm and identified effects have formed the basis of a number of new projects.
5F. Knowledge transfer-2 The trait identification work has been described in a number of outreach events such as JIC field walks. It forms the basis of an ambitious RCUK funded event for National Science and Engineering Week (6-15th March 2009) in which 6th form students will score a subset of the population for growth habit (winter v spring types) and map the gene controlling this trait back to the population using PCR based gene specific markers.

Objective 06 ***Exploiting T. monococcum*** **(RRes)** - a model for detection of traits, genes and variant alleles and for identifying phenotype: genotype relationships

6A. Objectives For this study a collection of 263 *T. monococcum* accessions was assembled at RRes from global germplasm centres and its genetic diversity assessed with molecular markers in comparison with hexaploid wheat. This diploid species was assessed for resistance against the major UK wheat diseases caused by Septoria tritici , two eyespot species, soil-borne cereal mosaic viruses, aphids and the take-all fungus, yellow rust, brown rust and powdery mildew. Variation in some other key traits was also explored such as variation in grain hardness and salt tolerance. Many genetic mapping populations were generated to understand the genetic basis of these novel traits either within this project or in collaboration with others. We also generated several *T. monococcum* – hexaploid wheat introgression lines, in order to produce pre-breeding materials for the transfer of the novel traits into UK commercial breeding programmes.
6B. Methods & Results A number of research methods were employed in this research. For novel traits identification, a combination of field and controlled environmental assessment were used. For genetic diversity assessment, SSR, NBS profiling, and DArT markers were used. Classical mutagenesis and breeding approaches were deployed to generate mutations, mapping populations and introgression lines. Association study and linkage mapping was used to identify novel genes, variant alleles and establish phenotype : genotype relationships. JoinMap and MapQTL were used for genetic linkage map construction and QTL mapping. All analyses were statistically explored using the required methods, for example, chi-square test, clustering analysis, similarity test, Komogrovo-Simoniv test for randomness and ANOVA with logit transformations for all the disease score data from the field experiments. One of these *T. monococcum* analyses already published in Jing et al., (2007) will soon appear as a detailed working example in a new statistics text book by Sue Welham, Salvador Gezan and Andrew Mead. Both *T. monococcum* and *T.*

monococcum – heaxploid wheat crossing protocols has been establish and will soon be available from the WGIN website as a step-by–step pictorial guide.

6C. Discussion of results The results achieved considerably exceeded the original objectives. This is reflected by the number of accessions collected (263 vs. 100), the molecular markers used (92 SSR markers and 860 DArT markers vs the 2 SSR markers per chromosome originally envisaged), wheat pathogens scored (8 species vs. 4 species), additional traits studied (grain quality and salt tolerance), generation of EMS and ion-beam mutagenised populations as well as over 20 mapping populations at F_2, F_3 and F_4 stages, various introgression lines so far taken to the F1BC1 generation, identification of a Septoria tritici blotch resistance locus that confers immunity to infection, identification of sources of resistance to the take-all fungus and the establishment of new inter-institute, national and international collaborations.

Many of the results obtained had been repeated at multiple sites and in multiple years of field trials, and / or have been reproducibly tested under controlled environment conditions (e.g. resistance to septoria tritici blotch and talk-all). Some of the traits have also been genetically verified by identifying the controlling genetic locus (e.g. *TmStb1* for resistance to *M. graminicola* isolate IPO323) (Jing et al., 2008). Overall, the results indicate that *T. monococcum* is a very rich source of novel traits and trait variation which has so far not been identified in hexaploid wheat. The diploid genome also greatly simplified the genetic analyses and we were impressed how well the SSR markers derived from the A genome of hexaploid wheat worked for the analysis on this species.

6D. Implications Scientifically, the main implications of the findings are: (1) *T. monococcum* is a rich source of novel traits for wheat genetic improvement; (2) *T. monococcum* is a good model for gene discovery and functional analysis; and (3) comparative studies between diploid and hexaploid wheat is an effective approach to dissect the molecular genetic mechanisms controlling important agronomic traits. Furthermore, as a result of the research, the UK wheat community had gradually realised over the last 5 years the importance of *T. monococcum* and started to use this species in their own research. In addition, the UK wheat breeding companies, most of who were originally extremely sceptical about the value of the research proposed using *T. monococcum,* have either started to voice their support for research on specific traits from *T. monococcum,* for example Nickerson / Advanta and RAGT for the *TmStb1* gene conferring Septoria resistance and/or are starting to use this species and the lead accessions we have identified to screen for traits of interests, for example, RAGT for eyespot resistance and CPB Twyfords for resistance to aphids.

7E. Future Work The findings generated within WGIN I formed the basis for a number of new projects: These were as follows: (a) A one year Rothamsted International Fellowship 2004 jointly with the Vavilov Institute St. Petersburg; (b) A PhD project to dissect genetic basis of resistance to talk-all (joint funded by BBSRC-HGCA, started Oct 2008 for 3 years; (c) Within the EU FP6 Intergrated Project ENDURE, a two year study to examine the selection pressure of *R* combinations on *M. graminicola* populations, and (d) The introgression of novel traits into hexaploid wheat to continue in WGIN.

7F. Knowledge transfer This has been carried out through the five years via a number of routes. *T. monococcum* accessions have been requested by national and international groups and also research protocols, results and

seeds were distributed to requesters and posted on the WGIN website. At Cereal 2006, 2007 and 2008, *T. monococcum* plots were demonstration plots, posters and stands were displayed and discussed with many farmer, farm advisors and others in the various AgIndustries. All the *T. monococcum* seed stocks are now available through the JIC genetic resources portal after an active collaboration with Mike Ambrose in 2008. The collaboration with the company Triticarte Australia has lead to the joint development of a new multi-*Triticum* species DArT chip which will be very useful to the international wheat community for association genetics studies. Finally, several *T. monococcum* – wheat introgression lines are in the pipeline to deliver to breeding companies as pre-breeding materials in 2009/2010 at the F1BC2 stage. Three peer reviewed publications have already appeared, a further one is under review and others are planned.

Objective 07 Mutagenesis **(RRes/JIC)** *To generate and make available knock-out and change of function mutants as a resource for gene discovery and the identification of novel allelic variants*

7A. Objectives-1 (RRes) All the tasks in this objective were completed, and were taken well beyond the targets originally planned. In addition to developing mutagenised populations of cv. Paragon at JIC, RRes also prepared a heavily mutagenised population of cv. Cadenza. This population was developed as a resource for reverse genetics, particularly Cel1-based TILLING. However, we have also collaborated with Dr. Mariann Rakszegi at the Hungarian Academy of Agricultural Sciences in Martonvásár to carry out further development of this resource. Field-based phenotyping on successive generations has identified a range of phenotypes in characters. In addition, the population now fixed at the M6 generation has been made freely available, and other groups have screened for traits such as starch properties (Kay Denyer, JIC).

7B. Methods & Results-1 RRes selected cv. Cadenza for mutagenesis, as this is a spring variety (and therefore does not require vernalisation for propagation), is a parent of the WGIN reference doubled haploid mapping population and is the standard cultivar used for transgenesis. We used ethylmethane sulphonate (EMS) as mutagen as it targets dG residues in DNA and wheat is strongly GC-rich in the coding regions of genes, thus increasing the number of target sites. Furthermore, GC-AT transitions are more than three times as likely to introduce stop codons into coding sequence as AT-GC transitions. In mutagenesis of diploid species such as barley, mutagen dosage is critical, as too low a dose results in a low mutation rate that is too low for efficient screening, while too high a dose results in a large proportion of sterile M_1 plants, presumably due to mutations in essential genes that give lethal phenotypes at the haploid gametophyte stage. As bread wheat is hexaploid, however, we argued that recessive, mutations in individual genes would be complemented by homoeologous copies on the other genomes, and therefore we chose to use a very high concentration of mutagen. We first tested a range of EMS concentrations and determined that overnight treatment with 0.6-0.9% (48-72mM) EMS allowed normal (if somewhat slow) germination and development of 60-80% of seeds.

We carried out full-scale mutagenesis over two years to create the wheat population. For the first batch we treated ~10,000 individuals by imbibing overnight (16h), half in 0.6% EMS and half in 0.9% EMS. After treatment,

seeds were washed in water and hand drilled in the field in spring. Here germination was considerably lower than under glass (~40%). After growing to maturity, single ears were collected from approx. 3,000 M_1 plants. A second batch of seeds was treated with EMS as above and planted in soil-based growing medium under glass, in 5x5cm cells as used for single seed descent. Plants were again grown to maturity and a further 3,000 M_1 ears collected.

The M_2 generation was created by planting a single seed from each M_1 ear, four to a pot and the pots arranged in a 6x4 block. The ears were labelled and stored under controlled humidity at 6°C and the pots were similarly labelled to give unique identifiers for each line, of the form CADx-y-z, where x = batch number (1 or 2), y = block number (1 to 30) and z = grid reference (A1 to H12). Thus, a total of 5,760 M_1 lines were established, although some failed to germinate. Tissue samples for DNA extraction were taken at the three-leaf stage: approx 5cm of young leaf material was harvested into the appropriate well of a deep microtitre plate and stored frozen at -75°C. DNA was extracted using a method kindly provided by Dr. Peter Jack (RAGT Seeds Ltd) and also stored at -75°C. At maturity, obvious phenotypes were noted and the M_3 grain was harvested, labelled and archived under controlled humidity at 6°C. The viability of this seed has remained close to 100%.

Although only obvious phenotypes were observed at M_2, this population has been grown in collaboration with the EU FP6 intergrated project "Healthgrain" at the Martonvásár site of Hungarian Academy of Agricultural Sciences. The population has been grown in the field in successive years at M_3, M_5 and M_6 and once under glass, by single seed descent, at M_4. A range of qualitative and quantitative phenotypes have been described, including leaf colour, flowering time, stature, ear morphology, fertility and grain colour, size and shape. A portion of the M_6 grain is archived at RRes and is generally available (although we have to charge a small fee for labour costs of sampling and of shipping). The population has already been distributed to Hungary, as described above, and to the University of Tuscia, Italy.

As well as DNA screening for mutations in specific genes by TILLING, we have also collaborated with outside laboratories for alternative screening methods. Part of the library has been screened by Dr. Domenico Lafiandra at the University of Tuscia for loss of Starch Granule Protein I (SGP1) by isolation of starch granules from grain followed by SDS-PAGE. The three isoforms of SGP1 have different molecular weights and migrate as discrete bands, and it was possible to identify lines with missing bands for each genome copy. In each case DNA sequencing showed the presence of mutations that explained the loss of the encoded protein. In collaboration with Dr. Rakszegi in Martonvásár we have also screened the population for white-grained mutants (Cadenza is a red-grained variety but contains only one functional copy of the *R* gene thatdetermines seed coat colour). Four white lines were identified and these form the basis of a BBSRC proposal to investigate the link between grain colour and dormancy.

7C. Discussion of results-1 This population is an extremely valuable resource for both forward (phenotypic) screening and reverse screening by TILLING or related technologies. As described below (Objective 9), the frequency of mutation observed in the population is approximately 40% - i.e. nearly half of all GC residues in wheat have been mutated somewhere in the population, which numbered about 4,500 at M_2. Thus a wide variety of mutations, including those

involving amino acid substitutions, premature translation stops and altered RNA splice sites, would be expected to be present. Screening platforms have been established using a variety of strategies, and these are available to outside users by collaboration. Alternatively we can supply limited quantities of pooled DNA samples for screening in users' laboratories, although it should be noted that the Cel1-based TILLING protocol is difficult to establish and requires specialist equipment.

7D. Implications-1 (see below - 7D. Implications-2)

7E. Future Work-1 The Cadenza EMS population is a long term resource that, in tandem with the TILLING screening in Objective 9, has already spawned multiple collaborative projects and collaboration – these will be detailed under this later objectives. One collaboration connected with the population is the EU Healthgrain project whose aim is to reduce the risk of metabolic syndrome related diseases in Europe by increasing the intake of protective compounds in whole grains. Within this project the Cadenza population is being used as a source of novel variation in bioactive components such as fibre and starch. We also intend to screen the population for variants in grain size and shape under a CSI project coordinated from RRes, whose aim is to investigate the link between wheat grain shape and milling yield.

7F. Knowledge transfer-1 The mutagenised Cadenza population has been distributed to several European research groups. In addition, DNA from the M_2 lines has been sent to the JIC Genome Centre, Norwich, as positive controls to aid them in establishing TILLING in wheat. White seed-coat mutants identified within the Cadenza population form the basis of a BBSRC Agri-Food proposal that has the backing of UK wheat breeders. Within this planned project, backcrossing and evaluation of mutant lines will be carried out by breeders including RAGT and KWS.

Andy Philips has spoken about the Cadenza population and the different TILLING platforms at numerous events. These are listed under Objective 9F.

7A. Objectives-2 (JIC) At JIC the development of over 7000 fixed EMS mutant lines and the optimisation of gamma ray irradiation to produce a population of 430 deletion lines in the spring variety Paragon are outstanding achievements of WGIN, representing some of the best resources of this nature available to the world wheat community.

7B. Methods and Results-2 Mutagenesis was achieved using Ethane methane sulphate (EMS) and gamma Irradiation. At JIC populations were genetically fixed by single seed descent (SSD) and field phenotyping was carried out on duplicate single plant rows each 1m in length.

7C. Discussion of results -2 A wide range of mutant phenotypes were scored systematically and notes were taken when new phenotypes emerged. Grain was archived and distributed as for the other germplasm collections. Field scores were validated in subsequent experiments.

7D. Implications-2 The availability of point mutations and deletions in an elite UK spring has opened new opportunities for the dissection of phenotypes relevant to UK conditions. Traits such as alternative dwarfing, altered senescence profiles, and increased plant biomass can be investigated using subsets of these lines. Because large quantities of fixed material are available for each mutant line the mutant phenotypes can be assessed on a whole replicated plot, rather than single plant, basis.

7E. Future Work-2 These materials are very widely used in a number of high profile projects and breeding programmes. Examples include- BBSRC-INRA NUE, BBSRC CSI grain shape, Defra LINK low phyate project, INRA (Clermont-Ferrand) reduced tiller work.
7F. Knowledge transfer-2 WGIN mutant material was the focus of the NIAB-JIC display at Cereals 2007, numerous field walks, and seminars at JIC.

Objective 08. ***Wheat Crosses*** This activity was not done after discussions with the commercial wheat breeders during project year 1.

Objective 09. *TILLING (RRes) To identify gene sequence variants with biological relevance.*
9A. Objectives The aim of this aspect of the core project was to establish TILLING methodologies through which mutations in specific genes could be identified within the mutagenised populations described in Objective 7. These objectives proved to be more technically demanding than had been anticipated, and staff changes mid-way through the project also were disruptive. However, the objectives of establishing the technique and generating novel sequence variants in genes of commercial significance were achieved.
9B. Methods and Results At the start of the project a novel method for reverse screening of mutant populations of Arabidopsis had been developed at the University of Washington in Seattle. The basis of the technique is the recognition of mismatched bases in heteroduplex double-stranded DNA by an enzyme from celery, Cel1. In order to establish the protocol we used an existing mutant in the *Rht-D1* gene. This proved to be a difficult target as parts of the gene are very GC rich, a recurring problem with wheat TILLING targets. Homoeologue-specific Rht primers were developed and tested for specificity using wheat aneuploid lines lacking each of the three genomes in turn. We successfully demonstrated that we could detect the *Rht-D1b* allele using TILLING with both double-stranded cleavage followed by fragment separation on agarose gels, or by single-stranded cleavage of fluorescent-labelled heteroduplexes and separation of polyacrylamide sequencing gels.

To detect efficiently the labelled cleavage products we successfully applied to BBSRC under the Research Equipment Initiative for part-funding to purchase three Licor 4300 Genetic Analysers. In addition, this grant also funded the purchase of a Beckman Biomek FX Liquid Handling Robot which was required for DNA quantification, normalisation and sample pooling.

To establish TILLING for novel mutations in wheat we first established a mutagenised population of cv Cadenza (described in Objective 7) and generated DNA samples from leaf material of each of the ~4,500 M_2 plants. The Biomek robot fitted with an automated DTX fluorimeter was used to quantify DNA concentration in these samples using fluorescence assay with picoGreen dye (Invitrogen), and to rearray the samples in 96-well plates to exclude those samples with DNA concentrations below a threshold level. Two-fold pools were then created for large-scale screening by TILLING: while detection of mutations in deeper (4-8x) pools is possible, this requires subsequent de-convolution to identify individual lines and at high mutation frequencies is not efficient. As Slade et al. (2005) had already shown proof-of-concept in wheat by mutagenesis and TILLING in the *Waxy* gene, rather than work on the *Rht* gene we refocused our efforts on identifying mutations in other

GA signalling components that would generate novel alleles to both understand control of stature in wheat and to generate useful germplasm.

We next investigated the question of whether genome-specific amplification was necessary to detect mutations in target genes in wheat. The three genomes of wheat are very similar, particularly within coding regions of genes, and the design of genome-specific primers can be problematical. To test whether TILLING was feasible with primers that would amplify all three genomes, we designed primers for *GAMyb*, encoding a transcription factor involved in GA signalling. DNA sequencing identified a total of 6 inter-genome polymorphisms in the 347bp amplified fragment from *GAMyb*. TILLING using these primers in a collection of 2x pooled samples from the Cadenza population showed that each of the intergenomic polymorphisms gave a Cel1 cleavage product, but this made individual point mutations very difficult to detect. All subsequent TILLING assays were therefore carried out using primers specific for each homoeologue; unfortunately, for GAMyb this proved impossible due to the low degree of sequence polymorphism between the three genes.

In addition to Cel1-based TILLING, we also conceived a mutation screening method based on high-resolution melting (HRM) of double-stranded DNA. Heating DNA in the presence of saturating amounts of dsDNA-specific fluorescent dyes such as LCGreen; differences in DNA sequence results in alteration of the melting profile, detected by changes in fluorescence. This requires the use of a sensitive plate-reading fluorimeter with fine temperature control; after promising tests with the Idaho Technology Lightscanner, the bought this instrument using Institute funds. We found that mutation detection using the Lightscanner was at least equally sensitive to Cel1-based TILLING, and the reduced target size (resulting in lower throughput) was compensated by reduced time and complexity of the protocol. These promising results allowed us successfully to apply for a one-year grant from the BBSRC Tools and Resources Development Fund, within which we further investigated the technique and developed it as a screening strategy. In particular, HRM is useful for targets with small exons and larger introns, where conventional TILLING with long (>1kbp) amplicons detects mainly intron mutations of no value.

The total number of genes screened by TILLING by core staff or by trainee visitors within the WGIN project is listed in Table 1. A number of other genes were investigated as targets but were rejected on the basis of either (i) too little pre-existing EST or genomic sequence data, (ii) having multiple paralogues with ill-defined roles or overlapping expression patterns (eg. *CKX*), (iii) too little difference between homoeologues, resulting in non-specific amplification (eg. *GAMyb*) or (iv) inefficient amplification from wheat genomic DNA.

Table 1: Numbers of confirmed mutations identified in target genes in hexaploid wheat by TILLING

Target gene	**Homoeologue**		
	A	**B**	**D**
GA20ox1	17	8	24
GID1	6	nd	nd
SbeIIa	nd	15	nd

SGP1	8	2	5
IsoA	16	15	nd
PMS2	3	nd	nd

9C. Discussion of results TILLING in a number of target genes has allowed us to estimate the nucleotide mutation frequency within the Cadenza EMS population to be 0.003-0.004%. This equates to 30-40 mutations per million base pairs. Thus, screening over a 1kbp region of any gene within the 4,500 lines in the collection should allow the identification of 135-180 mutations, suggesting that nearly half on all G/C residues have been mutated. This allows the identification of all types of mutation including those involving amino acid substitutions, premature translation stops and altered RNA splice sites would be present within any given gene. Therefore not only do we almost certainly have access to full knockout mutants for all genes, but also an allelic series varying in strength.

So far, no single point mutation identified by TILLING has been linked with a clear mutant phenotype, even when present in homozygous form. This was expected, as the homoeologous copies of any given gene are likely to complement, at least partially, the loss of one copy. It is therefore likely that mutants in each homoeologue will need to be identified and crossed together to allow the loss of all three homoeologues to be assessed. We have identified a double mutant containing likely knockouts in the GA20ox1A and GA20ox1B genes, but this also has no clear GA-related phenotype. It may be that in other cases, where one or more homoeologues are absent or expressed at low levels, single mutants may have phenotype. It should also be noted that as each mutated line carries up to 500,000 additional mutations, unrelated phenotypes are frequently visible, and backcrossing with non-mutagenised Cadenza to reduce the mutational load will be necessary before phenotypes can be assigned to the target genes.

9D. Implications The Cel1-based TILLING method has proved successful in identifying mutations in all target genes for which we developed genome-specific primers. However, a considerable amount of optimisation was often required for each target, and significant variation in the quality of results between runs was observed. When combined with the necessity of identifying and crossing mutants in each homoeologue, and also backcrossing to remove unlinked mutations, the effort required would suggest that while the TILLING approach may be extremely useful as a source of novel alleles in genes of proven utility, it may be inappropriate as a platform on which to test hypotheses, which might be better approached by, for example, an RNAi strategy.

9E. Future Work The mutagenised population, archived DNA samples from all M_2 plants and libraries of pooled samples ready for TILLING will be maintained as a resource by RRes. Those wishing to screen the population for novel mutations may do so by collaboration – normally through a hosted visit to the site – or may request pooled DNA plates. The facility is also being used within a number of BBRSC- and EU-funded project based at RRes and other UK institutions. The JIC genome centre also offer a TILLING screening service within which it should be possible to screen the Cadenza population.

Those mutant lines identified within the WGIN project continue to be exploited within core and BBSRC-funded projects. For example, mutations in *GA20ox1* are being tested for their effect of PHS sensitivity within the HFN LINK project jointly funded by Defra and BBSRC. Similarly, mutants in starch biosynthesis are being further investigated by groups at JIC and at the University of Tuscia in Italy.#

The WGIN TILLING programme also led to the establishment of a mutagenised population and mutation detection platform in durum (tetraploid) wheat, under the EU Optiwheat programme. This is aimed initially at drought resistance for Mediterranean countries but has created a resource of some 4,500 lines of durum wheat with similar mutation frequencies to those described for the bread wheat population above. The presence of only two homoeologues in durum wheat reduces the need for extensive crossing to observe phenotype and this may emerge as a compromise platform for functional genomics.

9F. Knowledge transfer Several point mutations identified within the TILLING project have been, or are about to be, transferred to UK breeders and other British and European laboratories. *GA20ox1* is a candidate gene for both pre-harvest sprouting resistance and for control of stature in wheat. In particular, *GA20ox1A* underlies a major PHS QTL that is conserved across several crop species. The mis-sense and splice sites mutations identified within this homoeologue will be tested by RAGT Seeds and KWS Seeds, partners in the HFN LINK Project, for effects on PHS in order to validate this association. Construction of molecular markers for the mutations is in hand at RRes, after which the wheat lines will be transferred to the breeders for back-crossing and evaluation. Similarly, crossing of different *GA20ox1* mutants will be carried out by the breeders to examine the effects on both PHS and height. Lines containing mutations in starch biosynthetic genes form part of industrial platforms for starch manipulation based at JIC Norwich (the Smart Carbohydrate Centre) and the University of Tuscia, Italy, and mutant lines have been distributed to these groups. The TILLING platform will also be used by two further project within the BBSRC Crop Science Initiative.

Andy Philips presented the work of the TILLING component of the WGIN project to a large variety of audiences and visitors, including: BBSRC wheat genomics meeting, INRA, Clermont (April 2005); TILLING workshop, Gatersleben, Germany (June 2006); PGEM meeting, Tenerife, Spain (Sept 2006); EU Healthgrain meeting, Lleida, Spain (October 2006); U. Keil, Germany (March 2006); CAAS Beijing and Huazhong Agricultural College, Wuhan, China (Dec 2007), AAB Meeting, Warwick (April 2008); PHS Meeting, Okayama, Japan (May 2008) and to students of Nottingham University and Writtle Agricultural College (2005-2008).

In addition, we have offered training in TILLING to a number of individuals from the University of Tuscia, Italy; Croptailor AB, Sweden (oat); EU Optiwwheat programme (scientists from Libya, Morocco, Italy and Tunisia); and Huazhong Agricultural University, Wuhan, China (oilseed rape). Future training visits planned include those from CAAS, Beijing (June and October 2009).

Objective 10 Analysis of rice-wheat synteny

10A. Objective This objective has been achieved fully. The development of gene based markers and their placement in genetic maps has facilitated the alignment of key wheat genes with rice, *Brachypodium distachyon*, and *Brachypodium sylvaticum*.
10B. Methods and Results The methods applied overlap with a number of WGIN outputs. They are bioinformatic eg whole genome homology searches of wheat EST assemblies against rice pseudomolecules and molecular approaches; the development of single strand conformation polymorphism (SSCP) assays for the region of interest. Genetic maps and deletion lines developed within WGIN are then used to assign the markers to the locus.
10C. Discussion of results The protocols that emerged from WGIN have proven to be robust. They have been applied to a number of major projects including the positional cloning of the major pairing locus in wheat (*Ph1*) and the alignment of the *Rht8* semi dwarfing gene to it's equivalent region in rice and Brachypodium.
10D. Implications WGIN has put in place tools and resources that give UK wheat breeding real leverage to exploit the genome sequences of rice, Brachypodium, and the emerging physical maps of barley and wheat.
10E. Future Work The methods developed will continue to play a major role in new research projects such as the fine mapping of the *Kr1* crossability locus in hexaploid wheat (Graham Moore, JIC).
10F. Knowledge transfer A number of colleagues (RRes, IAB, IBERS, RCP [Prague]) have visited JIC to share expertise in this approach. The work has been presented in a number of outreach events including a presentation to the East Midlands Farm Management Association in 2008.

Objective 011 Grain archiving
11A. Objective To make available grain from the diversity trial to researchers and / or projects outside the WGIN core project for further analysis.
11B. Methods and Results From each plot of the WGIN diversity trial (harvest years 2004 – 2008) 1 kg of grain was dried to 5 – 7 % moisture content, sealed in a plastic container and placed into a -20°C freezer. There are now over 4.500 samples stored along with the associated metadata.
11C. Discussion of results So far five research groups have accessed a selection of the grain samples for further analysis. Experiments in progress include grain shape and size, the relationship between grain architecture and milling properties, export of nitrogen and gene expression
11D. Implications Apart from the immediate value to researchers accessing grain for further analysis, their analysis will supplement existing WGIN data from the diversity trial and this is helping to enhance the value of the core project objectives. In addition, a field protocol has been devised to ensure that the samples collected through the combine harvester are pure and correctly dried to the same moisture content prior to storage at -20°C at Rothamsted Research.
11E. Future Work The availability of the grain samples will be advertised again in the May 2009 WGIN newsletter to encourage further use of this unique resource. The freezers and new walk in freezer room have an operational life of at least 20 years.

11F. Knowledge transfer The WGIN data on the diversity trial provides valuable additional information on the grain samples for any researchers conducting further trait analysis.

Objective 012 A WGIN Website

12A. Objective The website was created to raise the profile of the project and network as well as to make information on the project (general outline, results, events, contacts) accessible to all stakeholder groups and the general public.

12B. Methods and Results The website (www.wgin.org.uk) was set up using the php programming language. A website description was submitted to Google for improved website hits. Each page of the website consists of a header, a footer and an individual contents section. A style sheet was created to ensure uniform style across all pages of the website. The website was structured with a menu on the left and a menu bar across. The menu on the left includes buttons to access the key information. The menu across the top structures information according to interest groups and gives access to in-depth information. In order to facilitate browsing, a site map was created and a search function was installed. For easy access to new information a "New Additions" page was created, which appears on the homepage. A button to convert pages into a printer friendly version was included in the footer. To allow internal evaluation of website hits a web counter developed by Colin Denholm was installed in March 2007. This web counter was replaced by google analytics in June 2007. The appearance of the homepage was changed at regular intervals to expose new topics of interest and/or to highlight key activities within the core project. Changes and additions in line with Defra objectives were made according to stakeholder feedback and scientists/management requests. The WGIN website was linked to the OREGIN and BGIN websites as well as to the Rothamsted Research website and the BBSRC MONOGRAM website. The WGIN website prominently features both the defra logo and the WGIN logo on the homepage.

12C. Discussion of results There was regular feedback from stakeholders and the website evolved according to stakeholders needs. Scientists found it very useful to be able to direct enquiries on results (datasets in particular) to the website. Judging from a google analytics evaluation the project certainly gained higher visibility than would have been achieved solely through the rotated management meetings and the newsletter.

12D. Implications The website has helped to raise the profile of WGIN and to make it a "known" within the international wheat community. It has served the purpose of advertising meetings and allowing people to access information on past meetings. Data generated by the project has been accessed frequently through the website by UK and international users. Although initially, this open access to pre-published data caused problems, protocols are now in place to ensure that those accessing the WGIN data, acknowledge the source in grant proposal applications / review publications and also let WGIN know directly that this has taken place so that this information can be collated.

12E. Future Work By mid 2009, at the request of Defra, the WGIN website will be changed to the same format as the OREGIN website in order to harmonise the websites of the Defra GINs. The website in both the old and new versions will stay online to enable data accession. The new website at the same address

will be interlinked to the original website covering the WGIN project 2003-2008 to allow stakeholders to cross-reference project data.
12F. Knowledge transfer The purpose of WGIN and its website is to make scientific data, related information and activities available freely. Hence it is impossible to evaluate the full extent of knowledge transfer. We have however attempted to identify where WGIN data has already led to fruitful knowledge transfer by contacting known stakeholders within the UK. An overview of successful knowledge transfer can be accessed in the May 2008 WGIN Newsletter (http://www.wgin.org.uk/Stakeholders/WGINStakeholderNewsletterMay2008.pdf).

013 *Electronic Newsletter*
13A. Objective To present WGIN research data in a form that is accessible to many different stakeholder groups
13B. Methods and Results Nine newsletters have been produced in .pdf format and distributed to the stakeholder membership as well as posted on the WGIN website. The newsletters are also made available at the annual WGIN stakeholder meetings and other scientific outreach events that WGIN scientists attend, for example the Small Grain Cereals meetings, Cereals and Institute Open days. Generally the newsletters contain an introduction to the project and an update on research finding by project objective. The newsletter is also used to publicise outreach activities and to advertise the stakeholder meeting. Some recent newsletters focussed on a specific topic to raise awareness on certain aspects of the project, for example the resources generated, knowledge transfer and phenotype data.
13C. Discussion of results The uptake of the newsletter at the stakeholder meeting and outreach events has been good. The newsletter also receives a good distribution within the UK wheat community through the WGIN stakeholder membership list which now stands at 130 people / organisation representatives. There has been no analysis on how many people access the newsletter through the website.
13D. Implications The newsletter has proven a valuable periodical update on the WGIN project. It was also a useful medium to draw the attention to specific focus areas within the project
13E. Future Work We plan to continue the newsletter in the same format.
13F. Knowledge transfer The newsletters are another avenue of potential knowledge transfer employed within the WGIN project. There has been no specific assessment of how the newsletters in particular are aiding knowledge transfer.

Objective 014 ***Annual "Stakeholders' Forum"***
14A. Objectives To foster inter-connections between stakeholder groups, to increase general awareness of the WGIN project and its results and to provide more general information about the UK wheat crop and its uses.
14B. Methods and Results The six annual Stakeholder meetings (held each November) were each well attended (75-100 participants). Each meeting was advertised at least 6 months in advance on the website and by e-mail to the stakeholder mailing list, to CCFRA members, HGCA members and to members of the Rothamsted Research Association. The format of the meeting was to

give an overview of WGIN, updates on WGIN objectives and to include a group of presentations on a specific related subject by outside speakers. Plenty of time was left prior to, during and after the formal forum presentations for both formal and informal discussions between participants. The displaying of posters covering other UK wheat research activities was actively encouraged. For the last two meetings we have included talks covering the economic value of the global wheat crop and the UK position. All the presentations given at both the Management and Stakeholders meetings are freely available via the WGIN website. The stakeholder membership has increased steadily from about 60 in 2004 and is currently 131. Members include wheat breeders, farmers, agronomists, food and feed processors, members of HGCA and defra and researchers in the UK and overseas.

14C. Discussion of results The stakeholder meetings provided a good setting for publicising WGIN results and created an opportunity for informal exchange of ideas. Numerous newly funded projects have directly arise from these conversations.

14D. Implications The stakeholder meeting has raised awareness on WGIN within the wider community.

14E. Future Work The plan is to continue the stakeholder meetings in the same basic format, but with even greater time for questions and discussion.

14F. Knowledge transfer The forum has provided a good opportunity to publicise research findings to the stakeholders and also to put WGIN findings into context with presentations from specific stakeholder groups (e.g. grain quality, wheat farming, and economics).

Objective 015 To foster strong collaboration between UK wheat researchers

15A. Objective (as above)

15B. Methods and Results All of the WGIN management meetings were deliberately kept very open. The location was rotated to ensure good participation from all sites. Where a new technology was emerging from overseas or a new technology provider arrived in the UK , for example, DArT arrays in Australia and Idna Genetic Ltd, Norwich UK, these were give talking slots to explain their new advances. We also encouraged newly arriving PI researchers to the UK to attend and give some details of their research interests and to hear first hand about the WGIN project. For example, Dr Mary Byrnes (JIC, ex Cold Spring Harbour Labs, USA), Dr Rumina Ray, (University of Nottingham, ex University of Madrid, Spain) and Dr Cristobal Uauy (JIC, ex UC Davis, USA).

15C. Discussion of results This very flexible approach has lead to the inclusion of additional UK project partners to UK and European grant proposals and in two of the new LINK project.

15D. Implications The details of the timelines and delivery on all aspect of the core project were regularly scrutinised by others

15E. Future Work In the new WGIN project, the original style of management meeting will continue as a morning meeting. However, in the afternoon, the details of the timelines and delivery on all aspect of the core project will be regularly scrutinised by others. The stakeholder meetings are planned to continue in their existing format.

15F. Knowledge transfer There has been a lot and this has helped the initiation of many new projects involving researchers and PIs new to wheat. Overall the size of the UK wheat / cereals community has grown considerably over the past 5 years.

Objective 016 To foster LINK research projects

16A. Objective To re-connect public-private research partnerships on specific topics

16B. Methods and Results The tools and resources generated within the WGIN project were immediately made available to the wider wheat research community. Also most of the WGIN datasets were posted on the WGIN website shortly after these were generated. Three LINK projects have been funded to a total value of ~£1 million (see WGIN Newsletter, May 2008). All three arose directly as a result of discussions initiated at WGIN management meetings. In addition, of the 20 additional projects which have been funded as a direct result of WGIN (see WGIN Newsletter, May 2008)., 50% direct involve the involvement of the UK commercial wheat breeding community.

16C. Discussion of results There has been some use of LINK to fund follow up and new projects. However, the breeders and HGCA have insufficient funds to support a lot of LINK projects on wheat improvement. Of the £7.4 million in new wheat research funding attributable to WGIN, only £1million involves LINK.

16D. Implications LINK as it currently exists is underused for wheat improvement.

16E. Future Work To encourage other industrial sectors to join the wheat breeders to support wheat genetic improvement. Possibly to channel defra funds into supporting 4 year LINK PhD studentships.

16F. Knowledge transfer One of the main reasons for devising innovative and eye catching WGIN demonstration plots at Cereals 2006, 2007 and 2008 as well as widen the topics included at the annual stakeholder forum was to encourage the involvement of additional research sponsors. This has been successful, but the majority of the projects funded have not involved LINK funds.

Objective 017 To establish and maintain international collaborations.

17A. Objective (as above)

17B. Methods and Results Three proposal were written to the BBSRC under their ISIS scheme to fund the travel of up to 10 scientists to three overseas venues. Each was successful.

17C. Discussion of results International links have been strengthened as a result of joint workshops with CIMMYT (Dec 2004 and June 2007), INRA France (April 2005), and CAAS China (Dec 2007).

17D. Implications In the short term these WGIN activities lead to the exchange of ideas and key germplasm. In the longer term, they have formed the basis of successful grant proposals and PhD studentships.

17E. Future Work This type of activity will continue in the new WGIN project and a trip to locations involved in wheat breeding / wheat research in Hungary and /or the Czech Republic in 2009 are at the planning stage.

17F. Knowledge transfer A reciprocal UK trip was organised by CIMMYT staff in June 2007. This involved visiting the JIC, NIAB, University of Cambridge and

RRes sites. At both JIC and RRes the WGIN field trials were visited and the WGIN project discussed in detail.