



# Annual/Interim Project Report for Period **Feb 2015 to October 2015**

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## Project details

- Defra Project code: CH0106
- Project title: Wheat Genetic Improvement Network (WGIN) - Improving the resilience of UK wheat yield and quality through crop genetics and targeted traits analysis
- Defra Project Officer: Giulia Cuccato
- Name and address of contractor: Prof. Dr. Kim E. Hammond-Kosack  
Department of Plant Biology and Crop Science  
Rothamsted Research  
Harpenden  
Hertfordshire  
Postcode AL5 2JQ
- Contractor's Project Manager: Kim Hammond-Kosack
- Project: start date..... 20<sup>th</sup> February 2015  
end date..... 19<sup>th</sup> February 2017

## Objectives

7. Please list the 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 Officer giving the date of amendment.

Work Package A : Project management and finance [interim reports 1&2, finance report, Final report];  
Work Package 1: Maintain and expand a network of UK researchers and stakeholders. WP1.1: Maintaining and improving the WGIN platform through regular meetings; WP1.2: Maintain and update a website and add in new data types; WP1.3: Utilise the technologies available through collaborations (in UK and abroad) to maximise opportunities for UK wheat improvement; WP1.4: Regularly disseminate of WGIN results, new technologies and resources information to the wheat research community and the wheat industry, and improve the potential for industry exploitation (including via WGIN Newsletters); WP1.5: Establish industry-led forum to promote the uptake of newly characterised resources into associated projects.

Work Package 2: Maintain archive and produce new and freely-available information resources for genetic improvement of wheat. WP2.1 Ensure availability and distribution of all archive WGIN as well as newly produced genetic and genomic data; WP2.2 Production of new disease trait data making them publicly available via the WGIN information site, including yellow rust, brown rust, Septoria tritici blotch, powdery mildew (when present in the field) and two species of aphids; WP2.3 Exploring in detail the genetic components of key traits including exploring the Paragon NILs, drought tolerance, yield and quality resilience (NUE, grain N and C content, grain minerals, canopy longevity), improve the marker coverage on key mapping populations and key Watkins lines using of Breeder (BR) array, Improve the marker coverage for the genotype in the core Tm collection using the (BR) array; WP2.4 New QTLs development for each of the traits identified in WP2.3 with a LOD > 3.0.

Work Package 3: Maintenance of existing wheat lines and accessions and creation of new ones, exploring new traits. WP3.1 Establishment/maintenance of Avalon-Cadenza population and other new populations and trials; complete the two mapping populations (to F6) for T.monococcum for take-all resistance and genotype, complete the F2 mapping populations for T.monococcum for aphid resistance, genotype the Pargon x Garcia population, regenotype the entire A x C extended population held at RRes using SSR markers to confirm seed stocks following field multiplication WP3.2: Development of new near isogenic lines in wheat; create the next generation A x C population. Use the available A x C NILS to create a minimal TILING path of individual C segment introgression into a Avalon background and individual A segment introgression into a Cadenza background WP3.3: Develop new T. monococcum introgression for take all resistance and septoria resistance using key accessions; WP3.4: Quantifying variation in below ground traits and the function of the root system, including specific Paragon A x C NILs, Paragon x Garcia population and WGIN diversity (all 1<sup>st</sup> wheat crops) .

Work Package 4: Broadening the genetic base for wheat improvement. WP4.1: Continue to identify novel sources of genetic variation within wheat germplasm with UK sustainability prospective, reevaluate 10 accessions from the Watkins collection for resistance to multiple foliar fungal pathogens; reevaluate T. monococcum collection for resistance to the Warrior race of yellow rust, identify other suitable wheat collections that may provide new sources of variation for the traits of interest WP4.2: Classify germplasm variants for a catalogue of crop-relevant genes, using high-throughput screens, add relevant information into CerealsDB data archive; carry out exome capture on a priority list of genes to be developed in conjunction with the breeders and UK academics, for up to 96 wheat genotyped WP4.3: Establishment/maintenance of field trials for traits such as: NUE, drought tolerance, vigour or others identified by stakeholders; see WP2 for the traits to be evaluated via these field trials WP4.4: Development of gene-specific marker data for the new traits by combining the data generated in WP 4.2 with the existing and newly generated field phenotyping data from WP 4.3. WP4.5: Analysis of the genetic bases of trait variation, assess the effects of variant genes. Make available the A x C NIL null genotypes ( developed in WP 3.2) for subsequent physiological studies on any trait.

## Summary of Progress

8. Please summarise, in layperson's terms, 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.

WP1.1 Three WGIN management meetings have been held (Feb, July and Oct), one WGI stakeholder event was held (JIC in April) and another has been organised for Nov 2015. An application has gone in to BBSRC for a joint wheat workshop in Turkey in May 2016. WP1.2 The WGIN website has been updated with new datasets, the new project details and research partner details. WP1.3 The exome capture technology and the AxC TILING project has been explained and industry is now engaged. WP1.4 The newsletter is underway. WP1.5 The industry-led forum has been discussed with defra and the breeders but not yet implemented.

WP2.1 Specific seed stocks have been requested by x academics groups and 1 breeding company and this information has been captured in the master spreadsheet. In February 2014 (in WGIN 2) seed of five T. monococcum lines was sent to Limagrain, included take-all, aphid and septoria resistant lines.

The JIC has provided the following WGIN resources over the past 12 months:

Paragon x Chinese Spring	UK Academic
Paragon gamma	UK Academic
Paragon EMS	UK Academic
Paragon EMS	Dutch Academic
Subset Paragon Library	UK Commercial
Sunset Library Library	UK Commercial/Academic
Paragon Library	UK PhD project
Paragon x Garcia	Spanish Academic
Paragon x Garcia	CIMMYT
Paragon x Garcia	India Academic

WP2.2 Production of new disease trait data making them publicly available via the WGIN information site

Work Package 2.2 Screening germplasm for resilience to aphids.

Phenotyping studies were developed and conducted in the preceding LOLA and current WISP projects against the major aphid pests of UK wheat, the grain aphid, *Sitobion avenae*, and the bird-cherry oat aphid, *Rhopalosiphum padi*, (see Presentations from 04/03/15 WGIN3 Management Meeting on the WGIN website for full experimental methodology and results). Trials showed no aphid resilience in hexaploid elite or landrace collections, but more promising results with diploid species, particularly Triticum monococcum, where nymph production and survival were significantly reduced or absent on some lines. Crosses between resilient T. monococcum lines MDR045, MDR049 and MDR657, and the susceptible MDR037 were generated and the F1 populations were tested in the lab based aphid screening bioassay, against both aphid species. Analysis of results showed that *R. padi* mean nymph weight on the parental T. monococcum lines and crosses was not significantly reduced, and nymphs were produced on MDR045 and MDR657, which in previous screens had not supported nymphs. This could be due to the plants being older during this phenotyping screen. In contrast, *S. avenae* nymph weights on the same lines showed some differences, with a significant reduction on MDR045 and MDR049 and on the F1 plants from the 37 x 45 and 37 x 49 crosses, compared to the control. So there appears to be a separation in response between the aphid species on the T. monococcum crosses (see Presentations from 17/07/15 WGIN3 Management Meeting on the WGIN website for full experimental methodology and results). These plants will be taken through to the F2 generation and backcrossed to the susceptible line MDR037. Seed from the F2 generations and the backcrosses will be tested in the phenotyping screen and further generations produced. Plant material will be provided for genotyping.

See WP 4.2 for the field evaluations for the various foliar diseases

Work Package 2.3 Exploring in detail the genetic components of key traits

For the 2015 trial year, Yields, N and mineral contents of the 25 variety/4N level germplasm variety trial will be determined. Following harvest all samples have been processed and are under analysis. Yield data will be compiled for analysis in comparison to the previous seasons (yield and N back to 2004; mineral nutrients back to 2012). Anthesis and physiological maturity were scored manually, and senescence kinetics determined weekly by NDVI measurements (using a Tec5 radiometer). In addition samples were taken at anthesis to estimate biomass and N-uptake; this data will be used in conjunction with harvest data to assess importance of post-harvest N-uptake.

Subset (nine) of Paragon Library NILS including Rht's (Height), Ppd's (DTEM), Staygreen mutant and Lr19 alien introgression, plus Paragon (as populations that are developed furthest) have been grown (spring sowing) in three replicates of 6 m yield plots. Allowing confirmation of phenotypic effects for height, heading date, yield, thousand grain weight and grains per unit area.

In addition, newer NILs in Paragon for a wide of QTL and mutant loci were grown (autumn sown) in 1m<sup>2</sup> observation and multiplication plots.

The Paragon X Garcia drought trial was drilled at JIC (Oct)

WP3.1 Establishment/Maintenance of Avalon-Cadenza population and other new populations and trials. The two take-all T. monococcum mapping populations (MDR031 x MDR043 and MDR043 x MDR046) have been progressed by single seed descent in the glasshouse to the next generation (F5 stage). This will be repeated over the coming months to produce stable F6 seed for future field tests.

WP3.2 A x C TILING path. High density genotyping (Axiom 820K genotyping array) has been conducted on 18 of these NILs to show precisely which parts of the genome contribute these effects. A further 94 NILs have been prepared to extend this approach using the Axiom 36K breeders array.

In addition to Avalon x Cadenza and Malacca x Herward NILs, we have completed (up to field scale multiplication) the Paragon library of NILs. Nine of which have been drilled in replicated yield plots for detailed agronomic assesment.

Crosses have been made to produce new germplasm that captures the optimum combinations of the genes targeted by a selection of the above NILs.

A new WGIN population targeting the study of genes involved in water use efficiency has been multiplied and the first QTL analysis for agronomic traits is underway.

WP3.3: Develop new T. monococcum introgression.

One out of 60 embryo rescued hybrid wheat seeds grew to maturity (approx 3 x slower than self) but was sterile (male & female). Over 550 hybrid seeds are still available and some are in the process of being grown in the presence of different colchicine treatments in the next experiment. Two other introgression strategies have also been devised (see ppt MM Oct 2015)

WP3.4: Quantifying diversity in below ground traits and the function of the root system

A 25 variety, 4 N level, triplicated trial was set up for harvest in 2015. This was extended to a 30 variety trial for harvest in 2016.

Post-harvest 2015 soil cores were taken from all varieties in the WGIN Diversity trial at the 200 kg/ha N rate to phenotype for the take-all build-up trait. These samples will be processed and assessed over the winter months. This will provide valuable information on the consistency of the phenotype over different sites and seasons.

WP4.1: Continue to identify novel sources of genetic variation within wheat germplasm with UK sustainability prospective.

The RRes Triticum monococcum collection (263 accessions) was evaluated for susceptibility to yellow rust disease under field conditions. In the spring the trial was inoculated with three yellow rust isolates obtained from NIAB (Solstice isolate 08/21, KWS Sterling isolate 11/140 and Warrior isolate 11/08). A total of 216 accessions germinated and established successfully in the field and so were scored for yellow rust disease in late spring and summer 2015. The diverse T. monococcum accessions in the collection were all highly resistant/ non-host to yellow rust. Only two accessions, MDR634 and MDR288, showed low levels of sporulation on the flag leaf at flowering. Overall the non-host reaction of the whole T. monococcum collection to yellow rust restricts the use of the collection in follow-up association mapping studies. Instead work will focus on the more promising results from the Watkins foliar disease field trial (see below).

Ten Watkins hexaploid wheat landraces were sown in both a 1st wheat and 3rd wheat field trial to evaluate their resistance to multiple foliar pathogens in the presence of both low root disease (1st wheat) and high roots disease (3rd wheat) pressure. Yellow rust was the dominant disease that developed across the trial. Five out of 10 Watkins lines were highly resistant to yellow rust, including one line with no disease response (100% green leaf area) and four lines which showed some evidence of chlorosis and necrosis and only low levels of sporulation. All lines performed similarly in both the 1st and 3rd

wheat field trials, showing no evidence of an induced resistance response. Field crossing was carried out between these resistant lines and the fully susceptible spring wheat cultivar Fielder with the aim of developing mapping populations to characterise the genetic basis of resistance. The field trial will be repeated in 2016 field season to confirm the disease phenotypes. Full statistical analysis of the disease trait data from these two field trials will be completed in the autumn and then data will be made available via the WGIN website.

WP4.2 . Exome capture. The discussions at the WGIN stakeholder meeting and the July MM, focussed the project on the capture of sequence variation within the promoters of the A, B and D homoeologous loci. Genes will be nominated within eight trait categories, namely yield resilience, grain quality, biotic stress – fungi and insects, abiotic stress – drought, high temp, nutrient use efficiency, canopy development, flower biology and root architecture. A lead for each trait has been nominated (see ppt MM Oct 2015). The project is awaiting the latest wheat genome release into ENSEMBL (anticipated Nov 2015) before specific genes and their correct ID numbers are nominated by the breeders and UK academics.

WP4.3: Establishment/maintenance of field trials for traits such as: NUE, drought tolerance, vigour or others identified by stakeholders.

A 25 variety, 4 N level, triplicated trial was set up for harvest in 2015. This was extended to a 30 variety trial for harvest in 2016. This will be used to evaluate yield stability, yield components (particularly characteristics of canopy during grain filling), NUE and mineral uptake and partitioning.

Precise genetic materials (Near Isogenic Lines - NILs) from the Avalon x Cadenza WGIN population have been used to confirm the effects of eleven genes identified as quantitative trait loci (QTL) which control grain yield, crop height, and flowering time providing new insights into the way these traits interact. In turn this provides UK breeders with tools and knowledge to increase the yield stability of the UK wheat crop. Interpretation of these results together with climatological data has shed new light on genotype x environment interaction.

At RRes and JIC new phenomics tools have been developed to monitor the growth and development of all many WGIN materials, including a mini diversity trial ( 6 varieties) and specific NILs.

At RRes a subset of the WGIN Diversity trial varieties (six) have been sown at 3 N levels (omitting the highest WGIN N rate) in 3 replicate plots in the new Rothamsted Field Scanalyzer high throughput phenotyping system. Growth characteristics, growth stage, yield components will all be analysed with respect to N-inputs, funded as part of the 20:20 Wheat programme, and an overview of the output will be reported to the WGIN community. Cross comparison with data collected in the WGIN experiments will be made.

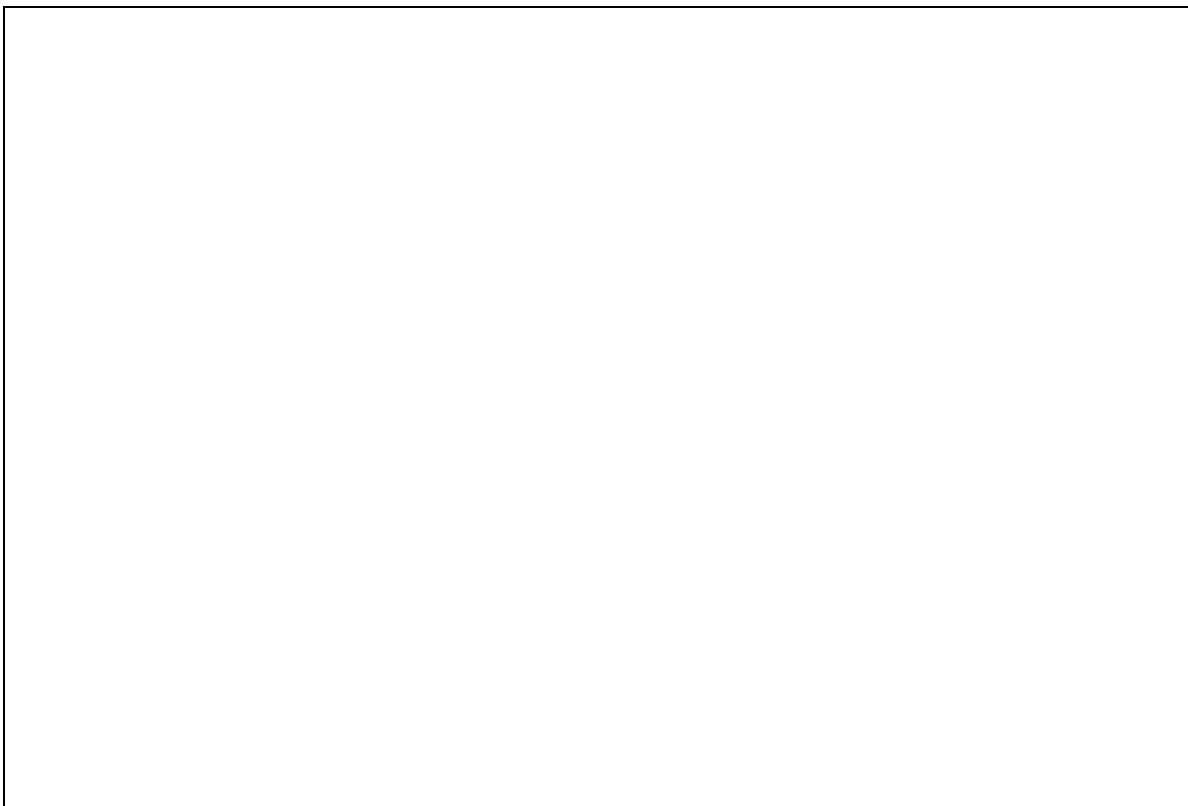
At JIC preparations were made for the autumn drilling of 7 lines from the subset Paragon Library NILS Rht's (Height) Ppd's (DTEM), plus Staygreen mutant and Lr19 alien introgression, under the Phenospex Plant Eye Laser scanning platform.

## Amendments to project

9. Are the current 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 objectives without the agreement of the Defra Project Officer.**



## Progress in relation to targets

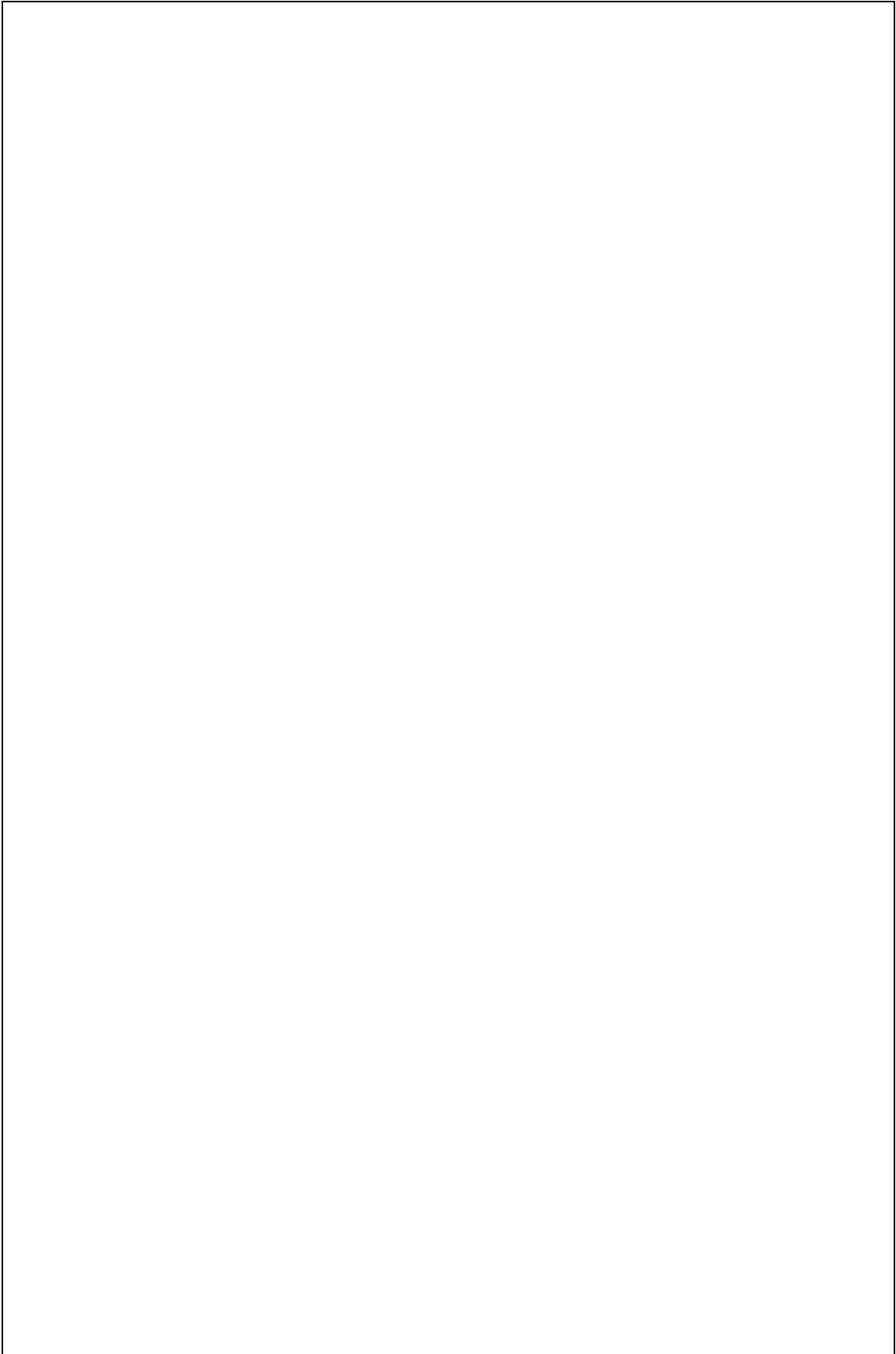
10. (a) List the agreed milestones for the year/period under report as set out in the contract or any agreed contract variation.

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
1	First stakeholder meeting	(March 15)	Yes	YES
2	Development of new near isogenic lines	(February 15)		
3	Further maintenance and distribution of Avalon x Cadenza doubled haploid population.	(throughout project)	Yes	
4	Genetic characterisation of Paragon mutants	(Feb 15)		
5	Identification of useful genetic variation in Watkins population.	(March 15)	Yes	
6	Development of new mapping populations	(Apr 15)		
7	First Interim written report to Defra	(June 15)	this report	late
8	Resistance to cereal aphids, information to establish the likely genetic basis of resistance to cereal aphid (Sept 15)	(Sept 15)		
9	Development of new QTL for yield at low and high N input	(Aug 15)		
10		(Sept 15)		

11	Information on stability of yield and nitrogen use efficiency parameters for elite varieties Collection of data on variation in canopy longevity and nitrogen remobilisation	(Oct 15)		
12	Evaluation of lines with good bread-making properties	(Dec 15)		
13		(Dec 15)		
14	Second stakeholder meeting	(Jan 16)		
15	Second Interim written report to Defra/Project evaluation	(Feb 16)		
16	Improvement of water use efficiency and drought tolerance traits	(Feb 16)		
17	Take-all disease, genetic basis, introgression of lines harbouring resistance Introgression of extreme resistance to Septoria tritici from T. monococcum	(March 16)		
18	Information on germplasm with new important traits.	(Apr 16)		
19	Grain Archiving: from each plot of the annual diversity and Avalon x Cadenza field	(Summer 16)		
20 and 21	Third Stakeholder meeting and 21. Report (Interim or final) (Dec 16)	(Dec 16)		

(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 publications

>Genome distribution of differential homoeologue contributions to leaf gene expression in bread wheat Harper, Andrea; Trick, Martin; He, Zhesi; Clissold, Leah; Fellgett, Alison; Griffiths, Simon; Bancroft, Ian, Plant Biotechnology Journal accepted

>Mauchline TH, Chedom-Fotso D, Chandra G, Samuels T, Greenaway N, Backhaus A, McMillan VE, Canning G, Powers SJ, Hammond-Kosack KE, Hirsch PR, Clark IM, Mehrabi Z, Roworth J, Burnell J & Malone JG (2015) An analysis of Pseudomonas genomic diversity in take-all infected wheat fields reveals the lasting impact of wheat cultivars on the soil microbiota. Environmental Microbiology, doi: 10.1111/1462-2920.13038 . This article was accompanied by a joint press release by the John Innes Centre and Rothamsted Research in October 2015 in which the WGIN project and defra was included.

Greenslade A. F. C., Ward J. L., Martin J. L., Corol D. I., Clark S. J., Smart, L. E. and Aradottir, G. I. Triticum monococcum lines with distinct metabolic phenotypes and phloem based partial resistance to the bird cherry oat aphid, Rhopalosiphum padi. Annals of Applied Biology (accepted)

### Oral and poster presentations

Kim Hammond-Kosack oral presentation on the WGIN project to MSc Plant Sciences Students University of Nottingham

>McMillan VE – Monogram conference @ RRes, April 2015 - poster presentation

>McMillan VE – Rothamsted Research Day, May 2015 – ppt on genetic control of take-all disease

>McMillan VE – Rhizosphere microbiome workshop @ RRes, August 2015 – ppt on LowTAB trait

>McMillan VE - INIA-Rothamsted workshop Uruguay, September 2015 – ppt on LowTAB trait

>6th May Nabim; Malcolm Hawkesford oral presentation on trials and stability work

>9th June CF Industries (USA) visit; Malcolm Hawkesford presentation of NUE and nutrient work

>10-11th June Cereals 2015 – displays and presentation, on Take-all LowTAB and root resistance traits ( V McMillan and Kim Hmamond-Kosack) and Aphid Resistance ( Lesley Smart)

>18/19 Wheat Initiative expert working group on NUE met at Rothamsted and visited WGIN field trials

>22nd Sept International Wheat Meeting, Sydney Australia; MJH plenary talk including WGIN trials.

>The WGIN diversity trials continues to be a major testing ground for UAV technology for crop/trial monitoring.

>MonoGram 2015 29 April- 1 May 2015 Gia Aradottir presented “Searching for insect resistance in wheat”

### New funding won

>Project Title: Aphid resistant wheat for the smallholder farmer in Africa

Funded by Technology Strategy Board: Agri-Tech Catalyst - Early Stage Feasibility - Round 4 due to start early 2016 Eligible costs estimated at £237,723; Rothamsted Research grant £71,923

Project title : Effector-directed approaches for improved disease resistance breeding in wheat. Funded by Syngenta £588,887. Due to start early 2016

- (b) Have opportunities for exploiting Intellectual

Property arising out of this work been identified?..... YES  NO

If YES, please give details.

>One to one discussion with commercial plant breeders during tours of WGIN field experiments were identified as two important ways to advertise the availability of germplasm resources and discuss new project ideas.

BBSRC LINK low protein bread making involves the use of Paragon library. JIC (Simon Griffiths) JIC PhD uses Paragon Ppd-1 and EMS stay green lines (Simon Griffiths).

International Wheat Yield Partnership uses Paragon biparental populations (Simon Griffiths and CIMMYT).

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

If YES, please give details.

## Future work

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

### Pathogen and Pest

Mapping populations developed in WGIN 3 between take-all resistant and susceptible Triticum monococcum genotypes will be an important resource for characterising the genetic basis of resistance and continuing introgression into hexaploid wheat. Similarly mapping populations developed between foliar disease resistant Watkins landraces and the modern spring wheat cultivar Fielder can be used to explore the genetic basis of this trait.

Phenotyping the WGIN Diversity trial for the take-all build-up phenotype generates additional trait information across different sites and seasons. In the future these long-term datasets will be important to model the disease-weather relationship to understand take-all epidemic development and explore the cultivar x site interaction. A PhD project has been devised and submitted into the University of Nottingham DTP 2015-2016 round (Vanessa McMillan, Kim Hammond-Kosack, Frank Vandenbosch and John Foukes).

Summer student project involving Lesley Smart and Gia Aradottir with student Amma Simon from Royal Holloway University entitled 'Arbuscular mycorrhizas influence Sitobion avenae feeding behaviour on resistant Triticum monococcum varieties'. This has since become a University of Nottingham DTP project (2015-2019) with the same advisory team plus Kim Hammond-Kosack.

### Nutrient Use efficiency

A PhD student from Professor Karling Muelings group at the University of Kiel will spend one year in the Hawkesford group at RRes and will investigate impacts of timing of N-application on yield and quality characteristic for a subset of the WGIN Diversity Trial varieties, utilising that experiment.

### Other activities

1. We will continue to use the Diversity trial as a test ground for UAV monitoring (additional project)
2. We will investigate impacts of timing of N-application on yield and quality characteristic for a subset of the Diversity Trial varieties, utilising that experiment (additional project)



## Declaration

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

Name

Kim Hammond-Kosack

Date

16<sup>th</sup> November 2015

Position held

Research Programme Leader