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Department
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Food & Rural Affairs

Evidence Project Final Report

- **Note**

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1. Defra Project code
2. Project title
3. Contractor organisation(s)
4. Total Defra project costs
(agreed fixed price)
5. Project: start date.....
end date.....

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(a) When preparing Evidence Project Final Reports contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

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(b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

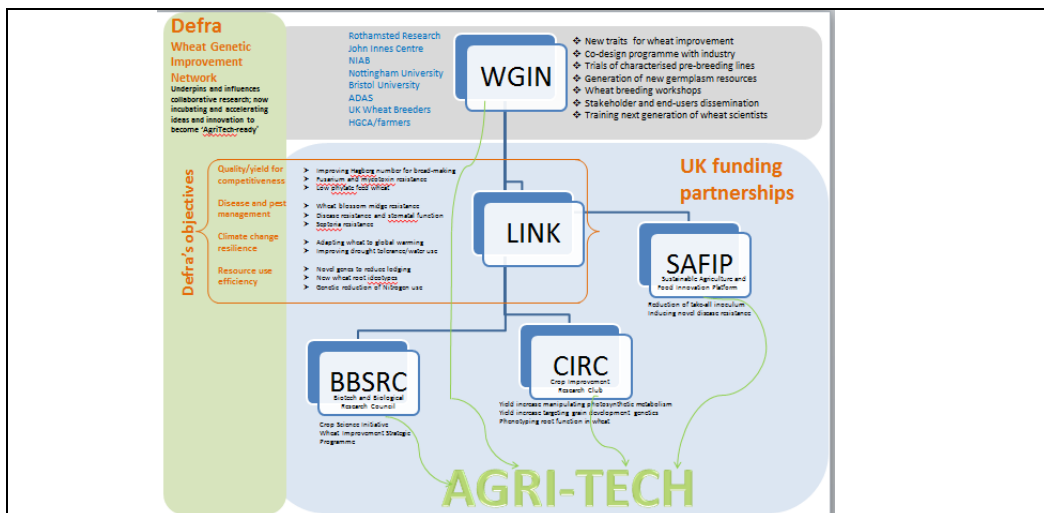
The WGIN Core Project started in 2008 and had two overarching aims. Firstly, the overall aim of the 'core' scientific project was to generate pre-breeding material carrying novel traits for UK breeding companies and to deliver accessible technologies, thereby ensuring the means are available to produce new, improved varieties. Within this integrated 'core' scientific project we combined and pursued collectively underpinning work on molecular markers, genetic and genomic research, together with novel trait identification and evaluation. The new genetic and molecular resources generated were made freely available for research in other Defra projects and for a wide range of wheat research projects in the UK.

The second overarching aim of WGIN was to maintain and further expand the wheat networking activities in the UK and overseas. The activities included regular management meetings, an annual stakeholder forum, newsletter focussed workshops in the UK and overseas and various types of outreach activities.

The project funded three partners (Rothamsted Research, John Innes Centre and the University of Nottingham) and support was allocated for two sub-contracted projects awarded to ADAS and the University of Nottingham through open competition in 2009. The research and the network were managed by a team that including the funded parties as well as representatives from all the key UK research groups and the nine UK based commercial breeders.

WGIN outputs were communicated to the wider scientific and end user communities, via a web site (www.wgin.org.uk), e.mail, a stakeholder forum, focussed meetings and peer reviewed publications.

Overall this 5 year project was highly successful. A legacy document was submitted to Defra in May 2014 detailing both the anticipated and unanticipated positive consequences of this project. Defra has summarised the wider outcomes and benefits of this project in the following Figure.



The Network

Over the 5 years a robust and inclusive network was maintained and further enhanced. Fourteen Management meetings held over the project duration at rotating locations which brought together wheat scientists and breeders. The five annual Stakeholder meetings (held each November) were also well attended (75-100 participants). At four Stakeholder meetings specific themes of high importance to the wheat industry were discussed and this involved an invited panel of experts. Topics included (1) exploring the reasons underlying the poorer performance of second wheats, and (2) examining the ever growing yield gap between breeders / national lists trials and on farm yields. All the presentations given at both the Management and Stakeholders meetings are freely available via the WGIN website (<http://www.WGIN.org.uk>). The WGIN 1 project website has also been updated and maintained. Over the project duration, the stakeholder membership has increased steadily from about 130 and is currently ~300. Members include wheat breeders, farmers, agronomists, food and feed processors, members of HGCA and Defra and researchers in the UK and overseas. Eight WGIN newsletters have been produced and distributed to the stakeholder membership as well as posted on the WGIN website. There have been four farm tours, two at JIC and two at RRes, providing opportunities to view WGIN field trials. These trials have also featured in the annual Rothamsted Research Association and John Innes Friends research day visits. International links have been strengthened as a result of joint workshops. Additional BBSRC funding was successfully won on six occasions to support joint wheat workshops with academic and industry based scientists in China, Brazil, India, France, CIMMYT Mexico, and Central and Eastern Europe. Several WGIN research demonstration plots were presented at Cereals 2009, 2010, 2012, 2014. These illustrated the genetic diversity in key new traits either identified or generated within the project. They also demonstrated their value to modern agriculture, in terms of environment protection, reduction of energy costs and improving crop resilience to the potential effects of climate change.

As a result of the sustained networking activities and making WGIN datasets available ahead of publication via the WGIN website, numerous new wheat genetic improvement projects have been funded which involve either joint public-industry funding or public only funded projects. These are summarised in the WGIN legacy document.

The Research

There have been several key research and resource highlights from the core WGIN project:

1. More than fifty new NILs were developed from the WGIN reference population Avalon x Cadenza. Most of these were reciprocal NILs between the parents. These contain QTL controlling height, heading date, and grain yield and two consecutive years of replicated field trials were completed at JIC using these NILs. These experiments validated 95% of the QTL discovered in WGIN 1. The NILs provide a unique data set for all to explore the effects of the introgressed chromosome segments on grain yield, yield components, height, heading date, and phenology.
2. A large number of additional NILs were produced in Paragon, Spark, Rialto, Cordiale, Napier, Hereward, Malacca and Robigus for semi dwarfing genes, grain shape, rooting, stem solidity, loaf volume, and bread cell size/density. These NILs could be phenotyped with a new round of WGIN funding.
3. Stocks of the Avalon x Cadenza mapping population and Paragon gamma and EMS mutant lines have been multiplied and maintained. Regular requests have been serviced for multiple stakeholders.
4. AE Watkins and Gediflux Germplasm Collections have been genotyped using SSR and KASP SNP

markers (DaRT was proposed originally). Allele mining in these collections has focussed on copy number variation (CNV) assessment using Taqman assays.

5. New segregating populations were produced by single seed descent for Paragon x Chinese Spring (international reference) and Paragon x JIC synthetic. Genetic maps have been produced for both populations using KASP markers.
6. To improve screening for variation in drought tolerance, isotope based methods were tested under UK field conditions. A positive linear relationship was demonstrated between grain yield and grain $^{12/13}\text{C}$ isotope discrimination ($\Delta^{13}\text{C}$) in two years of field trials on an 18 cultivar wheat panel under both fully irrigated (non-drought) ($R^2 = 0.41$, $P < 0.01$) and unirrigated (drought) ($R^2 = 0.70$, $P < 0.05$) conditions. This demonstrates the usefulness of this new technique for UK phenotyping.
7. A Paragon x Garcia mapping population contrasting for grain $\Delta^{13}\text{C}$ was developed for future drought research (F6 size ~300 lines developed by single seed decent). In collaboration with a BBSRC CiRC project a KASP SNP map was produced for this population.
8. Six successive trials primarily of, elite UK winter wheat germplasm ($n=47$ cultivars), investigated yield and nitrogen use efficiency (NUE). Consistent variation in NUE parameters was observed, sub-components were further explored and the underlying physiology was investigated. Information was also collected on the stability of yield and NUE parameters over multiple years for elite varieties and on variation in canopy longevity and nitrogen remobilisation during grain filling.
9. The Avalon x Cadenza mapping population was grown at two sites in multiple years, at both high and low nitrogen inputs. QTL for yield and NUE at low and high N inputs were identified.
10. Robust protocols were developed to screen wheat cultivars for resistance to infection by two aphid species using a clipped cage procedure under controlled environmental conditions.
11. Three elite wheat cultivar rotation trials revealed the significance of using the new low take-all inoculum build-up trait in 1st wheat crops to minimise take-all disease levels and thereby improve second wheat yields by between 0.2 and 2.7 tonnes/ hectare. In the 2nd year positive benefits to crop yield and overall root health were found to be cultivar independent (across all NABIM classes, $n=8$ cultivars).
12. Two accessions of diploid wheat species from the *Triticum monococcum* collection were confirmed through multiple years of field trialling to be potential source of tissue based root resistance to the take-all fungus. Two highly resistant accessions (MDR031 and MDR046) were identified and mapping populations were developed between susceptible and resistant accessions which are now at the F4 generation. The introgression of this trait into hexaploid wheat has also commenced with viable F1 seed and flowering F1 plants obtained.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
 - the objectives as set out in the contract;
 - the extent to which the objectives set out in the contract have been met;
 - details of methods used and the results obtained, including statistical analysis (if appropriate);
 - a discussion of the results and their reliability;
 - the main implications of the findings;
 - possible future work; and
 - any action resulting from the research (e.g. IP, Knowledge Exchange).

WGIN legacy document delivered to defra in May 2014.

PART 1 Overall outcomes and outputs achieved by the entire 10 year WGIN project

1. Regular interactions with all the UK based wheat breeding companies

A total of 32 management meetings were held over the 10 year period. On average 18 people attended each meeting with 50% from the wheat industry and 50% from academia. As a result of these regular interactions, the two communities have been connected and this has definitely influenced how the overall WGIN project has evolved. At these meeting there is a considerable dissemination of unpublished data and resources as well as discussions on emerging problems in the wheat crop. The full details of the participation at each meeting are given in [Appendix 1](#). As a direct result of these frequent meetings, the wheat breeders maintain, regularly review and discuss collectively a list of priority traits for wheat improvement. This has led to the inclusion of new trait topics in the WGIN2 project and to the development of additional wheat projects funded by other sponsors.

2. Generation of new germplasm resources, distributed throughout UK academia and industry and the additional 'in field' use of the WGIN trials

Major new germplasm resources were generated in WGIN including mapping populations, near isogenic lines, mutagenised populations, novel collections of hexaploid and diploid wheat and molecular markers. A total of 80 requests were made by UK and overseas academics and industry for genetic stocks, grain samples, markers or access to a specific WGIN trial to take measurements and / or additional samples. All requests were fulfilled. In [Appendix 2](#), these requests are summarised. Many of these requests have already led to either peer reviewed publications and / or new funded wheat projects on additional traits.

3. New knowledge disseminated via peer reviewed publications

Eighteen peer reviewed publications have arisen directly from the funded project. Interestingly, the number arising from wheat studies involving other sponsors is higher at 23 articles. This latter success demonstrates the high level of additional impact that has been achieved by others through the immediate use of the newly generated WGIN resources. Collectively, these papers cover a wide range of topics and have been produced by academics based at seven UK universities, one overseas university, three UK institutes and one overseas institute. Since ~~then~~ additional peer-reviewed articles have been published.

Comment [JS1]: Since when?

4. New unpublished knowledge disseminated to various types of academia and industry stakeholders

A stakeholder distribution list containing in excess of 300 individuals has been maintained throughout the project. In addition up to 8000 individuals are contacted annually via HGCA, NABIM and NFU distribution lists inviting them to the annual Stakeholder event, and to read the latest newsletters and new contents on the website. During the annual Stakeholder event any PI who had won funding for a new wheat project is invited to attend and to give an oral presentation. The WGIN e-mail is used regularly for specific queries and enquiries.

5. Community led discussion on emerging topics of collective interest to the wheat breeding community

Since 2010, as part of the annual Stakeholder event, through joint breeder-academic consensus we have selected a topic for discussion by an invited panel of experts. The topics covered were 'UK wheat field yields lagging behind breeders projections (2010), Successful 2nd and 3rd wheat crops and issue of Take-all root disease (2011), Emerging disease patterns (2012), and Yield and quality stability (2013). Each of these discussions has identified many gaps in our current understanding of the wheat crop performance, the stability of key traits, the underlying genetic and mechanistic basis of many traits and the potential inter-dependence of key traits.

6. Collective discussion and exploration of new traits for wheat improvement

The WGIN project has provided a 'launch pad' for several newly appointed PIs and existing

PIs and research teams to start work on trait improvement in bread wheat and to join the UK wheat research community. Projects that have been successfully initiated using the newly generated WGIN resources include improved photosynthetic capacity, heat stress at crop anthesis, resistance to aphid infection and studies of grain development and composition including spatial patterns of protein distribution and increased grain protein content.

7. Training the next generation of wheat scientists

This is a major unanticipated outcome of the WGIN project. A total of 20 PhD students have directly benefitted from the ongoing field experiments, genetic resources and stored grain samples provided by WGIN. Nine PhD students have already graduated. These 20 students have been registered at six UK Universities and 2 overseas Universities (China and Hungary). Five of the main supervisors receive no direct funding from WGIN. In addition, there has been considerable opportunity to train undergraduates and MSc students (17 students) and overseas visitors (10 students) in wheat research by involving them in field experimentation.

8. Organising a series of 'open invitation' overseas workshops linking UK academics and industry to relevant international wheat communities.

Additional BBSRC funding was successfully won on six occasions to support joint wheat workshops with academic and industry based scientists in China, Brazil, India, France (x2), CIMMYT Mexico (x2), and Central and Eastern Europe (x2). Typically these workshops were attended by up to 15 UK wheat academics, three to five UK wheat breeding companies and a similar number from the participating country / countries. The main benefits have been the sharing of information, exchange of resources, exchange of personnel for specific training and the writing of joint grants and publications.

9. Generating considerable new income for wheat research in the UK

A total of 47 new wheat research projects using one or more of the new resources generated within WGIN have been funded. The new funds raised for wheat research are in excess of £40.3 million. This new research includes topics already under investigation within WGIN but also 28 projects on new topics. Within these new funds, £11.0 million has been gained for 31 projects lead by PIs not sponsored within WGIN. A wide range of funding agencies have become involved, including BBSRC, HGCA, Defra, EU, European Research Council, the wheat breeding industries and various charities. Most projects are either three or four years duration, whilst three are of five years duration.

10. Representatives from non-EU countries and the EU commission coming to find out about how this government sponsored public-private project works.

11. Acquisition of ten continuous years of field data on key traits using commercially relevant wheat cultivars including yield, nitrogen use efficiency and soil health.

Unpublished data is provided by request or via the password protected WGIN Wikispace site which is accessed by the nine UK based wheat breeding companies.

12. Ten consecutive years of stored grain samples from the varietal diversity field trials linked to detailed weather data, which could be explored for the stability of multiple traits.

This carefully archived grain resource obtained from the annual WGIN diversity NUE trial is now vast, in excess of 8000 samples, 500g in size, one from each plot, stored at minus 20°C. So far very few groups have actively used this unique sample resource and the associated metadata. Potentially, many projects could directly benefit by including these samples in their analysis. The vast range of weather experienced over the cropping years would be extremely valuable when exploring the stability of any grain trait between cultivars and seasons.

Summary of core research results for Objective 2 through to Objective 12

Objective 2 – Production of Near Isogenic Lines

Within WGIN 2 more than fifty new NILs were developed. Most of these were reciprocal NILs between the parents of the WGIN reference population Avalon x Cadenza. These were for QTL controlling height, heading date, and grain yield. This experiment culminated in two consecutive years of replicated field trials, each with 1800 entries of 6m² 'yield' plots in a 3x replicated randomised block design. These experiments validated 95% of the QTL discovered in WGIN 1. The NILs provide a unique data set for all of the effects of the introgressed chromosome segments on grain yield, yield components, height, heading date, and phenology.

In addition to the Avalon x Cadenza NILs a large number have been produced in Paragon, Spark, Rialto, Cordiale, Napier, Hereward, Malacca and Robigus for semi dwarfing genes, grain shape, rooting, stem solidity, loaf volume, and bread cell size/density. These NILs could be phenotyped with a new round of WGIN funding.

Objective 3 - The Avalon x Cadenza mapping population

The multiplication and distribution of the AxC population has continued. A repeating cycle of regeneration from single plant glasshouse sources for which wheat ears are enclosed to prevent cross pollination has ensured very high seed purity for stakeholders. Glasshouse and field quality assurance has been achieved by genotyping, observation, and scoring traits of high heritability.

Objective 4 - Paragon gamma and EMS mutant lines

Stocks of these populations have been multiplied and maintained. Regular requests have been serviced for multiple stakeholders.

Objective 5 - AE Watkins and Gediflux Germplasm Collections

WGIN has continued the multiplication and maintenance of these materials together with the BBSRC WISP team who have exploited these resources to a high level. Both collections have been genotyped within the WGIN programme using SSR and KASP SNP markers (DaRT was proposed originally). The Gediflux collection has been extensively phenotyped, work which led to the funding of a PhD studentship by Limagrain. Allele mining in these collections has focussed on copy number variation (CNV) assessment using Taqman assays.

Objective 6 - New Mapping populations will align WGIN with the international wheat genome sequencing effort

New segregating populations were produced by single seed descent for Paragon x Chinese Spring (international reference) and Paragon x JIC synthetic. Genetic maps have been produced for both populations using KASP. Outside of WGIN collaborators have conducted RNA based genotyping by sequencing 48 individuals of the Paragon x CS population to produce a very high density SNP maps.

Objective 7 - Insect resistance in wheat: Cereal aphids

Assessing the differential susceptibility to two cereal aphid species of targeted lines from the Spark x Rialto mapping population

The major cereal aphid pests of UK wheat, the grain aphid, *Sitobion avenae*, and the bird-cherry oat aphid, *Rhopalosiphum padi*, are both vectors for Barley Yellow Dwarf Virus (BYDV) a serious disease of wheat crops. *Sitobion avenae* has recently developed resistance to pyrethroid insecticides, the main chemical control agents used against these pests. The development of insect resistant wheat varieties would provide growers with a sustainable alternative to broad spectrum toxicants. Seventeen lines from the mapping population, produced from the crossing of UK varieties Spark and Rialto, were tested against *R. padi* and *S. avenae* in laboratory bioassays. Replicated groups of 10 alate (winged) aphids were given the choice between two seedlings, one of a standard variety, Solstice and the other of the test variety. The number of alates settled and the number of nymphs produced on each seedling was recorded at 24h. These data were then compared for statistically significant differences and the number of nymphs produced was expressed as a proportion of the nymphs produced on Solstice in the same assay, thus providing a "preference index" for both 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 was very susceptible to both aphids (Figure 1 shows results for *R. padi*).

***Rhopalosiphum padi* choice tests with Spark x Rialto mapping lines. Nymphs produced at 24h as a proportion of nymphs produced on Solstice = 1**

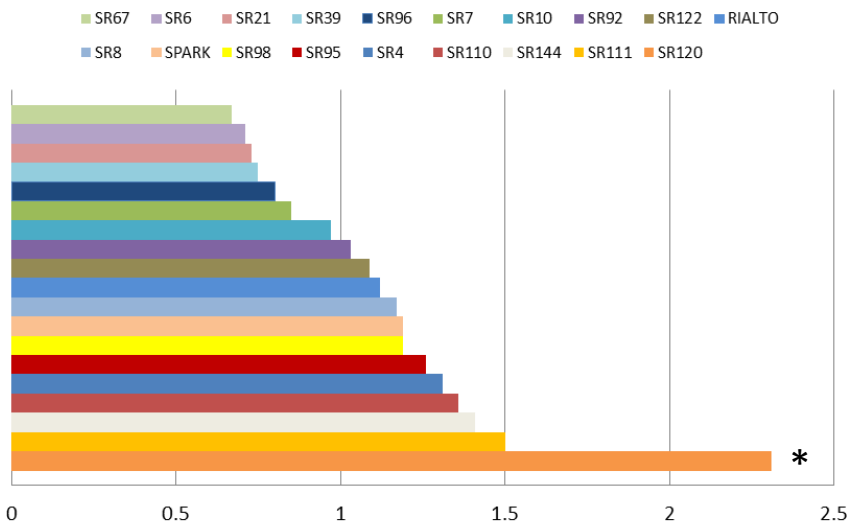


Figure 1 Preference of *R. padi* for nymph production on lines from the Spark x Rialto mapping population compared to Solstice

There were no obvious differences between the leaf surfaces of SR120, the two parental lines and Solstice when examined under UV high powered stereo microscope, which ruled out that particular aspect of leaf morphology from affecting aphid settlement.

More detailed developmental assays were performed for eight of the lines from the mapping population, chosen from the most and least preferred lines for each aphid species, plus the two parental lines and Solstice. Replicated groups of 5 alates were placed in clip cages, attached to leaves of test wheat seedlings and left overnight to produce nymphs. The neonate nymphs were weighed in batches of 5 on a microbalance and then transferred to the first leaf of 7-8 day old seedlings of Solstice, as the standard variety, or of the test line on which they were produced. At least 12 batches of nymphs were set up on each plant line and each batch was enclosed in a clip cage. Surviving nymphs were re-weighed in their batches after 7 days and the Mean Relative Growth Rate (MRGR) was calculated as:

$$\text{MRGR} = (\ln 7\text{day weight} - \ln \text{birth weight}) / \text{number of days (7)}$$

All data were subjected to ANOVA to determine any significant differences. The MRGR of the aphids on each line is presented as a proportion of the MRGR on Solstice in the same trial (Figure 2).

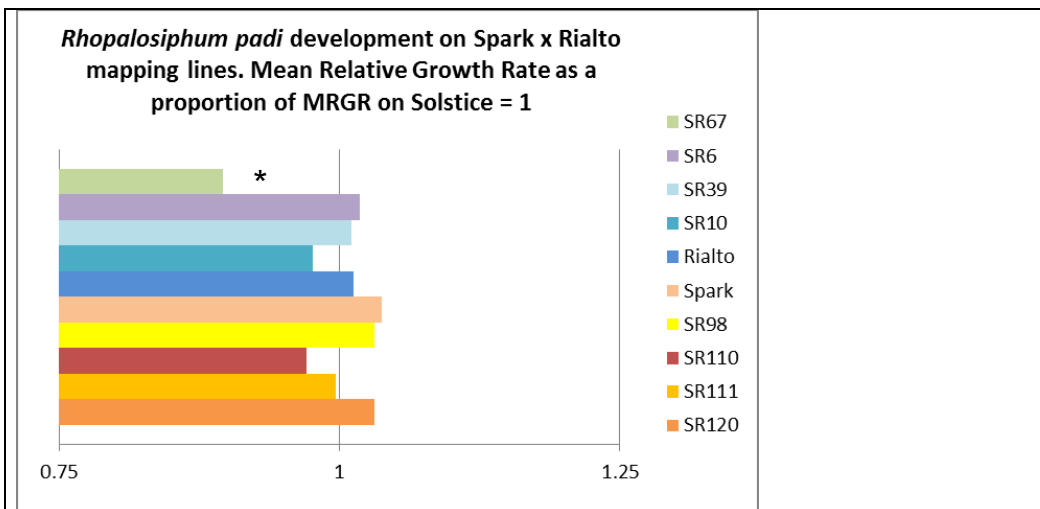


Figure 2. MRGR of *R. padi* on selected lines compared to MRGR on Solstice.

There were no significant differences between MRGR on the lines compared to Solstice for either aphid except for *R. padi* on line SR67. Excepting line SR67, settlement preference of alates did not relate to the development rate of the nymphs. These data will be 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.

Investigation of possible aphid resistance in hexaploid, tetraploid and diploid wheat lines was continued in PhD project "Aphid resistance in wheat varieties" (H. Elek) and in the BBSRC Lola project "Enhancing diversity in UK wheat through a public sector prebreeding programme". The one year WGIN project and the PhD project has led to the development of a robust protocol to screen wheat germplasm collections for resistance against aphids.

Objective 8 – Nitrogen Use efficiency NUE and NUE linked QTLs

Between 2009 and 2014, six successive trials with elite, primarily UK winter wheat germplasm, investigated yield and nitrogen use efficiency (NUE). The diversity of sub-traits contributing to these major complex traits was identified and many aspects of the underlying physiology investigated, including dissection of roles of individual genes in the N-remobilisation processes. Subsets of the germplasm were used for individual studies, for example by investigating the interaction of yield and quality. An initial assessment of trait stability was achieved with this dataset. This trial is also proving a useful test site for remote sensing using UAV technology (i.e. an Octocopter).

In parallel, the Avalon x Cadenza mapping population was grown at two sites in multiple years, at both high and low nitrogen inputs. Multiple QTL were identified, some of which align with other known traits and some new QTL require further investigation. High N and low N specific QTL were differentiated. Some field nitrogen acquisition QTL match QTL obtained in a laboratory based root QTL screen using the same population. Individual Avalon x Cadenza lines have been analysed for patterns of metabolite profiles in senescing leaves, facilitating building of a model of remobilisation pathways in response to nitrogen availability.

Key research results as bullet points

- Variation in NUE parameters for a diverse wheat germplasm set collected
- QTL for yield and NUE at low and high N inputs identified in the Avalon x Cadenza population
- Information collected on stability of yield and NUE parameters over multiple years for elite varieties
- Data obtained on variation in canopy longevity and nitrogen remobilisation during grain filling

Objective 9 - John Foulkes (UoN)- Tolerance to drought

A positive linear relationship was demonstrated between grain yield and grain $^{12/13}$ C isotope discrimination ($\Delta^{13}C$) over two years of field trials on an 18 cultivar wheat panel under both fully irrigated (non-drought) ($R^2 = 0.41$, $P < 0.01$) and unirrigated (drought) ($R^2 = 0.70$, $P < 0.05$) conditions. This demonstrates the usefulness of this technique as a phenotyping screen for grain yield under drought conditions in the UK. There was a significant negative linear relationship between flag-leaf $^{16/18}$ O enrichment ($\Delta^{18}O$) and grain $\Delta^{13}C$ amongst cultivars ($R^2 = 0.45$, $P < 0.01$) under irrigation, and the

combination of these two traits was found to be useful in identifying genotypes **combining high water use with high water-use efficiency under irrigated** conditions. Phenotyping of a Savannah x Rialto doubled-haploid population (94 lines) over two years under unirrigated and irrigated conditions and genetic analysis identified a novel QTL for grain $\Delta^{13}\text{C}$ under unirrigated conditions on chromosome 2A. A new single seed descent mapping population of 350 lines derived from a cross between Paragon and Garcia contrasting for grain $\Delta^{13}\text{C}$ was developed as a resource for future wheat drought research in the UK. In collaboration with a BBSRC CiRC project a KASP SNP map was produced for this population.

Objective 10 – Take-all disease

Robust sources of resistance to take-all disease have been identified within the Watkins and Gediflux hexaploid wheat collections (Objectives 10.1-10.4) and the diploid wheat species *Triticum monococcum* (Objectives 10.6 and 10.7). These provide a very valuable genetic resource for future work to introgress this resistance into elite hexaploid wheat.

Two putative major QTLs were identified which control the new take-all inoculum build-up (TAB) trait in elite hexaploid wheat (Objective 10.9). Cultivar rotation trials demonstrated a reduction in take-all disease and substantial 2nd wheat yield improvements by utilising this new genetic trait in the first year of wheat cropping (Objective 10.8).

Three elite wheat cultivar rotation trials (each 2 years in duration) revealed the significance of using the new low take-all inoculum build-up (lowTAB) trait in 1st wheat crops to minimise take-all disease levels and thereby improve second wheat yields by between 0.2 and 2.7 tonnes / hectare. In the 2nd year positive benefits to crop yield and overall root health were found to be cultivar independent (across all NABIM classes, n=8 cultivars).

Objective 11 - Introgression of resistance to *Zymoseptoria tritici* from *T. monococcum* into hexaploid wheat

The initial approach to cross directly *T. monococum* lines of interest onto various hexaploid wheat genotypes was successful. However the resultant F1 progeny recovered 20 days post-pollination by embryo rescue either failed to produce spikes or the spikes failed to produce pollen. Some reciprocal crosses were also tried, but these failed to set grain.

An alternative crossing strategy was chosen and so far this has proven to be more successful. F1 progeny were generated by crossing *Triticum monococcum* with the hexaploid wheat (*T. aestivum*) cultivar Paragon harbouring the pairing locus mutation *ph-1*. So far, five *T. monococcum* accessions have been successfully crossed to the Paragon *ph-1* line, and a total of 290 F1 seed have been recovered. Embryo rescue was completed and F1 plants have been recovered. The F1 plants came into flower in late Nov 2014 and have been used as the pollen donor in crosses to wild-type Paragon plants. There were no signs of hybrid necrosis.

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

Some inter-comparison of the traits measured in objectives 8, 9 and 10 has been done across sites and seasons, but this analysis was not completed within the project timeframe. Once all the 2014 data becomes available, further inter-comparisons will be done. The plan is to present the completed analyses at a special stakeholder meeting to be held at Rothamsted Research in April /May 2015, when there will also be the opportunity to visit the relevant field trials.

The outcomes of the WGIN networking objectives 13 through to 20 are summarised in the legacy document text already presented (see above).

MILESTONES - Summary of progress on the agreed project milestones.

In the Table, colour coded outcomes (right column) using a traffic light system have been used to indicate the progress achieved against the project milestones. Key: **green** indicates fully completed, **orange** indicates considerable progress has been made and **red** indicates difficulties were encountered and the activity is incomplete.

WGIN2 Milestones	Progress
Objective 1.1 Organise three management meetings during each project year, produce approved minutes and visit one the WGIN field trials at one site	

Objective 1.2 To establish the scientific strategic and research programme for the core project activities	Green
Objective 1.3 To ensure that all the UK wheat research community is aware of the resources emerging from the core programme	Green
Objective 1.4 To provide a central focus for wider studies of wheat genetic improvement in the UK, funded by BBSRC and other bodies	Green
Objective 1.5 To facilitate liaison between public sector research on the wheat genetic improvement and stakeholders' in the plant breeding and food industries.	Green
Objective 1.6 To represent UK research on wheat genetic improvement in the wider international arena.	Green
Objective 1.7 Responsible budget management	Green
Objective 2.1 Crossing programme advanced as far as BC 1 for yield, height, and heading date NIL development	Green
Objective 2.2 Fixed homozygotes produced for yield, height, and heading date BC2 derived NILs.	Green
Objective 2.7 Field phenotypes collected for yield, height and heading date NILs.	Green
Objective 3.1 Continue the maintenance and availability of authentic seed stocks of the Avalon x Cadenza population	Green
Objective 4.1 Twenty Paragon mutant x BIRST spring wheat F2s grown in the field	Green
Objective 4.2 Fifteen lines representing phenotypic extremes selected for BSA- DNA extracted	Green
Objective 5.1 AEWatkins crosses made for SSD. AE Watkins and Gediflux subjected to DArT genotyping, cladogram and core set.	Green
Objective 6.1 Paragon x Chinese Spring and Paragon x JIC Synthetic SSD populations self fertilised up to F6	Green
Objective 7.1 Differential susceptibility to cereal aphids of lines from Spark x Rialto mapping population determined	Green
Objective 8.1 Complete field experimentation on Av x Cad at low N inputs	Green
Objective 8.5 NUE and yield assessments on 24 lines at 4 N levels for selected germplasm (all years)	Green
Objective 9.1 Complete phenotyping and data analysis for drought tolerance traits in elite winter wheat varieties in 2009/10 &10/11.	Green
Objective 9.2 QTL analysis to identify genome locations associated with WUE and drought tolerance traits completed.	Red
Objective 9.3 Complete development of one new DH population in an elite modern background segregating for drought-tolerance traits.	Green
Objective 9.4 Association genetics analysis of drought tolerance traits using AE Watkins & Gediflux collections	Green
Objective 9.5 Collation of diverse germplasm collection (cultivars, advanced lines) from worldwide drought-tolerance wheat breeding programmes completed.	Green
Objective 10.1 To screen the AE Watkins Collection and an 'improved' Gediflux Collection to identify potential additional sources of resistance to the take-all fungus under field conditions	Green
Objective 10.8 Identification and characterisation of hexaploid wheat germplasm which reduce take-all inoculum build up (TAB) in soil, this will involve scoring the genotypes in the annual diversity trial as well as in a separate TAB trial and selected hexaploid genotypes based on pedigree analyses - in total 5 years of trials	Green
Objective 10.9 Explore the genetic basis of take-all inoculum build up using the Avalon x Cadenza mapping population - in total 3 years of trials using the full population and after 2 years of trials using a sub-set of the population containing and lacking the identified major QTLs.	Yellow
Objective 11.1 Convert the two closest linked DArT markers to the <i>TmStb1</i> locus to PCR based markers	Red
Objective 11.2 Complete the backcrossing of F1 plants to a range of hexaploid wheats and prepare detailed SOPs for this activity	Yellow
Objective 12.1 To interconnect the NUE and WUE a selection of genotypes will be included in at least 2 years of trialling	Yellow
Objective 13.1 Grain harvest from NUE diversity trial and A x C DH mapping population - all plots	Green

Objective 14.1 To procure one or more sub-contractor project through a formal application process	
Objective 14.3 For each subcontract to produce both interim and final reports at the indicated time intervals and for these to be placed on the WGIN website	
Objective 15.1 To maintain the WGIN website and include all results and new information	
Objective 16.1 To produce and circulate two electronic stakeholder newsletters	
Objective 17.1 to hold an annual stakeholders meeting in November	
Objective 18.1 To hold an open focused workshop on the Avalon x Cadenza mapping population	
Objective 18.2 To hold an open focused workshop on the archived samples from the 1st five years of the NUE diversity and A x C field trials and thereby identify existing and new projects which could use this resource	
Objective 18.3 To hold an open discussion with the wheat breeders which focuses on fulfilling crop vernalisation requirements for UK grown wheats	
Objective 19.1 To organise joint workshops each year with either CIMMYT, INRA, Adelaide and ICARDA by raising suitable funding from other sources	
Objective 20.1 To publicise the project on an annual basis through talks, field demonstrations, articles in the popular press and through interviews / discussions with interested NGOs and other organisations	

Legacy Document delivered to defra in May 2014

PART 2

Provide answers to the following three specific questions

- What will now be possible using the results of this work?
- What problems can now be solved?
- What avenues will be opening up as a result?

This information will be used by defra to indicate what can be built upon in the future, using various funding sources.

The ANSWERS

i) [What will now be possible using the results of this work?](#)

- **The improvement of many traits in wheat using a non GM approach.** This can be achieved by exploiting the variation that already exists in the germplasm collections, the mapping populations, and the various *de novo* speciality germplasm variation resources generated by WGIN. To improve a trait, the approach used by breeders and academics could be either phenotyping, genotyping or a combination of both.
- **Genetic mendelisation of new key traits of interest.** Within WGIN many new phenotypes have been mapped to one or a few major quantitative trait loci (QTLs). Subsequently, near isogenic pairs of lines (NILs), which either possess or lack the QTL of interest, have been generated by genotyping. To further explore the sub-components underlying each trait, these paired NILs can be used within WGIN and also distributed to others and carefully evaluated in replicated field trials and / or in glasshouse experiments. The characterisation of NILs in the future will permit fine phenotyping of the specific sub-component traits, which underlie very complex traits. For example, yield stability over sites and seasons, improved nutrient use efficiency.
- **Exploration of the interdependency of key traits above or below ground in multiple wheat genotypes.** These types of inter-comparative studies can be done immediately by using existing data sets across sites and seasons and by linking WGIN data sets to those generated in other wheat projects. These analyses can be used to pin-point wheat genotypes showing highly contrasting linked traits. This will provide commercially useable variation as well as opportunities for additional academic research on each trait combination.

- **The modelling of existing WGIN data sets to predict root health in future wheat crops.**

Ten years of take-all inoculum and disease are now available within WGIN. These data can be used to link weather data to the take-all inoculum build up trait in 1st wheat crops and the take-all disease development in 2nd and 3rd wheat crops. By this approach a model describing take-all development could be developed and refined. This model could then be used to predict for farms and crop agronomists the good and bad take-all years. This information would then be included within decision support systems and would influence crop species rotations.

- **The use of new molecular markers, instead of extensive field phenotyping, to fix each newly discovered trait in commercial breeding programmes.** This approach will help to accelerate commercial wheat breeding in the UK. The use of molecular markers will permit the rapid incorporation of any new traits identified in WGIN into commercial programmes.

- **The use of the WGIN grain archive to explore the stability of key quality traits.** Over 8,000 grain samples from 10 years of field trialling have been archived at minus 20^oC. These samples are from a wide range of modern and more historic wheat grown under four different nitrogen regimes. This grain archive could immediately be used to explore the stability of specific traits of emerging interest and to determine how stability is affected by N inputs. For example, the fibre composition of the endosperm, which influences the overall intake of dietary fibre from white bread consumption, alpha amylase content, which influences pre-harvest grain sprouting, or, asparagine content which directly influences acrylamide formation during the manufacturing of certain food types.

- **The use of the WGIN genotype collections to identify new sources of resistance to emerging problematic pathogens and pests.** Most pathogen and pest populations are continuously evolving either because of human intervention, human travel or changing climate. New pathogens and pests that have recently emerged to threaten the UK wheat crop include the Ramularia fungus, the yellow rust fungus and wheat bulb fly. Horizon scanning has identified the black stem rust fungus and Russian wheat aphid as possible problems in the near future. In addition, frequently occurring diseases, for example Septoria leaf blotch are re-emerging as problems to most wheat farmers due to the emergence of fungicide resistant strains. Within WGIN the considerable hexaploid and diploid genetic resources assembled could be screened in the future for potential sources of novel resistance.

ii) [What problems can now be solved ?](#)

- **Improving the stability of grain yield and grain protein.** Improving crop stability will raise farm profits, grow the UK rural economy and make best use of the crop inputs applied (i.e. fertilisers, PGRs and pesticides). In addition, the UK wheat grain user community will be able to purchase 'grain fit for purpose' more frequently from locally grown sources, thereby reducing transportation needs.

- **Improving root health to make better use of fertiliser inputs thereby increasing UK water quality.** Poor wheat root growth caused by root infecting pathogens (primarily the take-all fungus) leads to the incomplete use of applied fertilisers. These unused fertilisers can be leached away during periods of high rainfall into neighbouring water courses and in turn sea estuaries. Improving root function through the use of wheat genetics will reduce the risk of eutrophication in nitrate vulnerable regions of the UK.

- **Increasing total crop biomass.** Finding wheat genotypes that support a bigger crop biomass can in turn be used by commercial plant breeders to raise grain yields.

- **Altering the root-shoot index of the wheat crop whilst maintaining the harvest index.**

To improve wheat crop yield and overall yield resilience, the root and stem base systems also need to be significantly improved to ensure these potentially higher yielding crops are correctly supported. Improvements to root and stem base performance could be achieved by

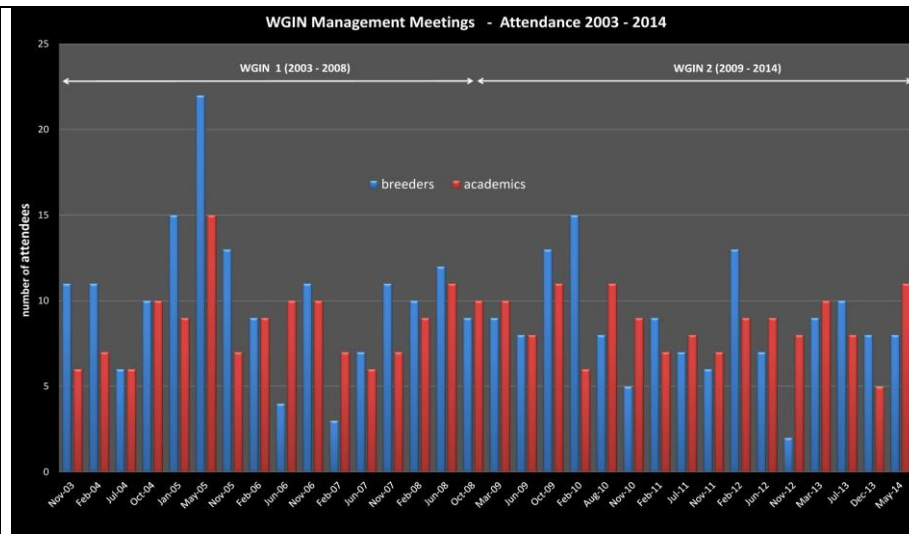
diverting some root biomass into more favourable types of root system development, e.g. slightly deeper rooting and by slightly changing the architecture of the stem base and / or the relationship between the stem base and the root system.

- **Alleviating transient crop stresses.** Over the last 10 years the UK weather has often become more extreme. Winter crops can be waterlogged for weeks which then can delay crop canopy development in the spring. At crop anthesis high temperatures and /or temporary drought can cause transient water stress with devastating consequences on final grain yield. Appropriate 'in field' protocols could be established to screen the WGIN germplasm collections for genetic variation in useful new traits that permit crop resilience to these different types of transient stresses.
- **Improved resistance to yellow rust, mildew, Septoria and take-all.** In the past, the entire WGIN *T. monococcum* collection was found to be totally immune to infection by yellow rust, mildew and septoria. Whereas only a few accessions showed a high level of resistance to take all root disease. This WGIN *T. monococcum* collection could immediately be included in screening for resistance against the new races of yellow rust that have appeared across Europe in 2014 and checked for continuing resistance to mildew, septoria and take-all. The use of this novel source of resistance for commercial wheat improvement is possible via the use of the *ph-1* mutant which permits the pairing of chromosomes between wheat species and therefore the exchange of genetic information.

iii) [What avenues will be opened up as a result ?](#)

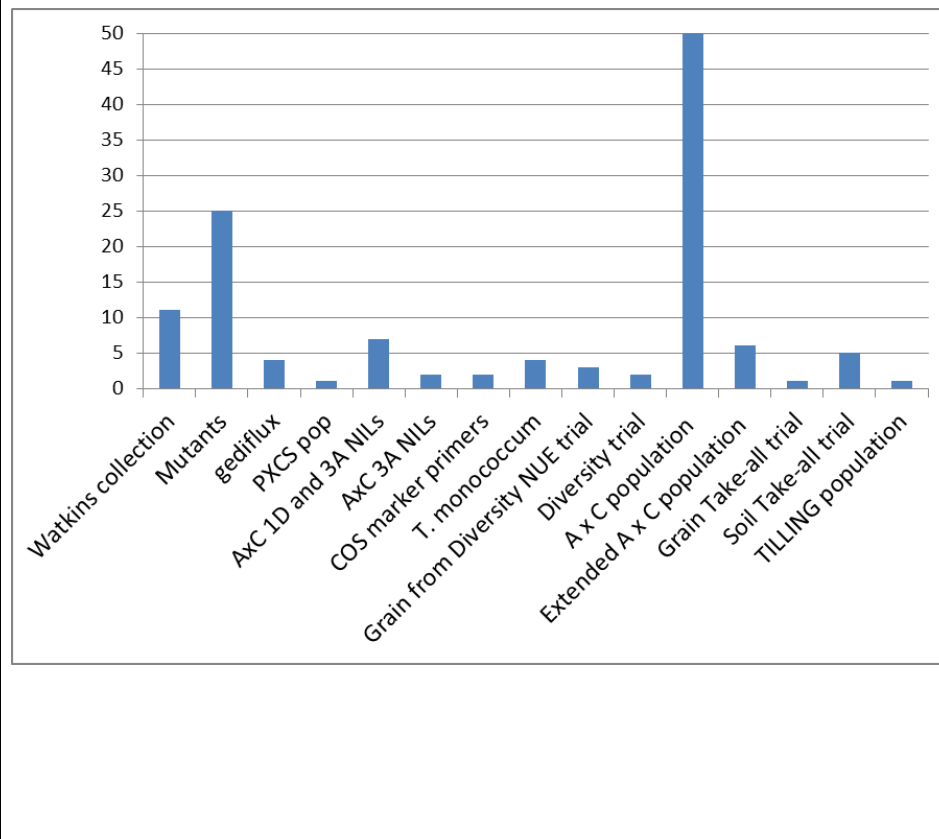
- **For any future new wheat traits of commercial or academic interest the lead in time will be minimal.** The WGIN resources can be accessed, the genetic diversity appropriately sampled and then phenotyped or genotyped for trait variation /gene variants and thereby improve any trait of interest. The use of WGIN resources could commence immediately in already awarded grants or form the basis of a new grant application. The entire UK commercial wheat breeding community has access to these new resources and new trait knowledge.
- **Exploring in detail the component parts of key traits.** During WGIN1 and WGIN 2, exploring the sub-components of a trait of interest was only infrequently done. However, with newly emerging protocols, the sub-sampling of plant tissues and new types of remote imaging many additional sub-traits could be explored. For example, detailed sampling of floral components at the key stages in floral and grain development or the use of remote sensing to measure quantitatively crop development on a weekly or daily basis.
- **Quantifying variation in below ground traits.** The function of the root system can be explored throughout crop development by inserting soil moisture probes and /or access tubes into the standing wheat crop and taking measurements on a daily or weekly basis. These data can be used to calculate the rate of water uptake at different soil depths throughout the season, for different wheat genotypes under stressed and non-stressed conditions. These types of remote below ground analysis could be applied to future WGIN trials and form the basis of a germplasm screen.
- **Removal of negative stress regulators that may be affecting wheat crop performance when growing under high input conditions.** The mutagenised wheat populations and the TILLING technique established in WGIN 1 and subsequently further developed in a BBSRC BBR grant, could be used in two ways. Either, to screen for plants that have produced greater biomass under unstressed or stressed conditions, or, through TILLING to identify a null allele for genes that are highly unregulated under stressful conditions, but which could reduce plant growth/development rates if expressed under high input conditions.

Appendix 1: Attendance of WGIN management meetings

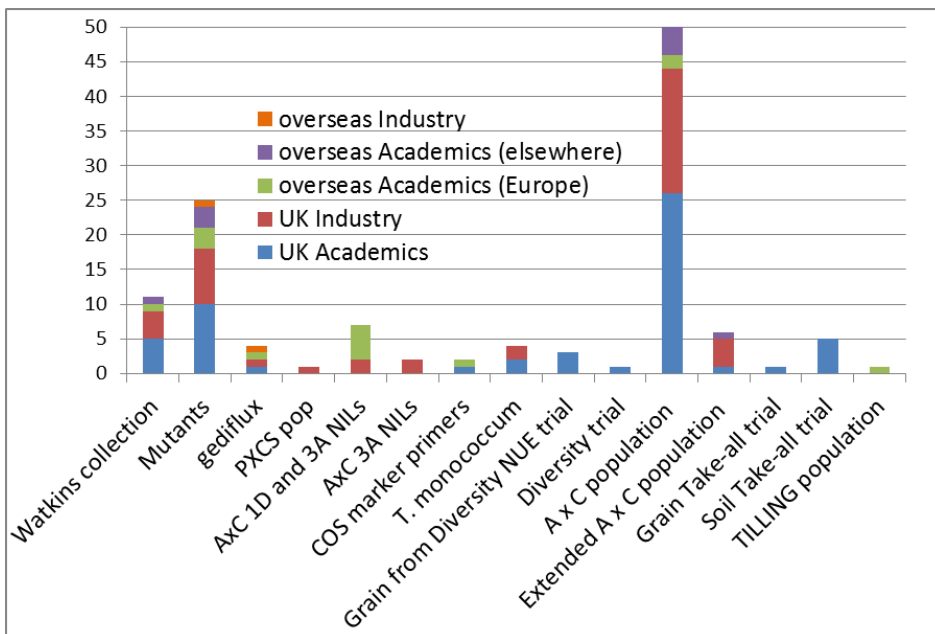


Appendix 2: Requests for WGIN resources

a) Requests for each resource (sum total 124)



b) Requests by academic institutions and industry in the UK and overseas



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9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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Articles in Popular Press

Hawkesford, M. J. 2012. The diversity of nitrogen use efficiency for wheat varieties and the potential for crop improvement. **Better Crops**, 12, pp. 7-9. <http://www.ipni.net/publication/bettercrops.nsf>

Cereals 2011 (June) Living display and three posters on take-all inoculum build up, root resistance in wheat to Take-all and the agronomic practices which can minimise the risk of Take-all. Prepared and presented by Vanessa McMillan and Richard Gutteridge. This display had immediate on line coverage of the take-all story on Farmers Weekly interactive online entitled 'Take-all levels linked to first wheat - 13th June 2011'

16.06.2009: Article in **Farmers Weekly** on Wheat Take-All (<http://www.fwi.co.uk/Articles/2009/06/16/116155/Wheat-take-all-research.htm>)

August 2009: The diversity trials were filmed as part of the **BBC report 'Averting a perfect storm of shortages'** (<http://news.bbc.co.uk/1/hi/world/8219184.stm>)

Article written for the **Next Generations Foods** website entitled '**WGIN - Improving the Environmental Footprint of Farming**' available at <http://www.nextgenerationfood.com/article/WGIN>

The WGIN *T. monococcum* field trial appeared in the BBSRC brochure '**The Bioscience behind: Secure harvests**' in the section 'Getting novel traits into wheat'.