Enhancing diversity in UK wheat through a public sector pre-breeding programme

Part 1A Previous research track record

This proposal brings together internationally renowned experts in wheat research with a unique range of skills covering genetics, genomics, physiology, biochemistry, breeding, QTL dissection and underlying mechanisms, to re-establish a pre-breeding programme in the public sector.

Programme manager:

Graham Moore: In collaboration with Prof Mike Gale's group, Graham Moore demonstrated the presence of conserved chromosome segments, 'synteny', within the genomes of wheat, maize, sorghum, millet and sugarcane based on the knowledge of gene order in the rice genome. Moore proposed and subsequently demonstrated the application of rice and *Brachypodium* as genome models exploiting, 'synteny', between these species and the wheat genome [1]. The synteny concept has had a major impact on cereal research strategies being widely used in the cereal academic and industrial communities in the UK and internationally. Publications relating to synteny are highly cited and in recognition of this work, Gale and Moore received the Royal Society Darwin Medal in 1998. Recently Moore's group has focused its research on understanding how chromosome pairing is controlled in polyploid wheats and their diploid relatives. Moore investigated the effect of a key domestication locus in wheat, namely *Ph1*, in collaboration with the cell biologist Peter Shaw. The molecular analysis of the *Ph1* locus in wheat was reported by Moore's group [2,3] (BBSRC grant BBS/B/11931). Although Moore's research has focused its research on hexaploid wheat and despite the difficulty of this biological system, his group have published 6 papers in Nature or its sister journals on biology of wheat. Moore has significant managerial experience including the management of BBSRC's Cereals IGF programme, a large and strategic research programme. Time commitment: 20%

Pillar Leaders:

Simon Griffiths ('Landrace Pillar' with John Snape). Simon Griffiths leads the JIC component of the Defra Wheat Genetic Improvement Network (WGIN) which has developed a number of the germplasm resources and methodologies directly exploited in the proposed programme. This includes the AE Watkins and Gediflux germplasm collections, and the use of Paragon as a UK reference genotype. Griffiths is co PI with Prof John Snape in several ongoing or recently ended projects studying strategic questions important for UK and global wheat production, such as nutrient use efficiency (NUE), end use functionality, new breeding systems, diffuse pollution, grain shape and stress tolerance. Successful new projects cover the development of new gene based marker systems, identification of novel variation for dietary fibre in wheat, the genetic control of biomass driving yield potential, the use of wheat straw for bioethanol production, and, with NIAB, the positional cloning of the alternative dwarfing gene *Rht8*. Griffith's skills span the gap between the focussed pursuit of detailed, fundamental, biological understanding as displayed by his role in the seminal characterisation of *Ph1* as a post doc, and his more recent interest in understanding and manipulating the genetic control whole crop phenotypes particularly in terms of adaptation, stature, and yield potential. Griffiths has extensive experience in the management of large programmes including WGIN, the projects already described, and the JIC in-house genotyping services. Griffiths has close ties and joint projects with all the major UK based wheat breeding companies and international centres especially CIMMYT and INRA. Time commitment: 20%.

Andy Greenland (Synthetics Pillar). Andy Greenland is research director at NIAB. He has more than 20 years experience in academic and commercial pre-breeding research. He joined NIAB from Syngenta in December 2005 and leads the pre-breeding Research at NIAB. His interests lie in the practical application of science to breeding including (with Ian Mackay) the development of highly recombinant mapping populations underpinning gene discovery (MAGIC), and the application of novel breeding methodologies, including genome-wide selection and GM. Working closely with all the major UK wheat breeding companies, he is leading the assessment of synthetic hexaploid wheats (SHWs) generated at CIMMYT as potential sources of useful traits for UK wheat improvement. Synthetic wheats are generated by artificially crossing tetraploid wheats with diploid

wheat progenitors. This approach broadens the pool of genetic variation relative to that in modern bread wheat which originally arose from a few chance hybridizations some 10,000 years ago. Time commitment: 15%.

Ian King (Alien introgression Pillar"). Ian King has over 26 years experience in alien introgression in the monocots and is presently the last expert in wheat/alien introgression in the UK and one of the last in the world. His research has included: the study and exploitation of gametocidal chromosomes in wheat to stabilise addition lines; development of a nuclear male sterility system for hybrid wheat production; development and exploitation of genomic *in situ* hybridisation in combination with genetic markers to characterise wheat/alien introgression lines; demonstration that *T. urartu* was the A genome donor of hexaploid wheat; production of salt tolerant lines of wheat and wheat/alien amphidiploids. In addition to wheat, he has also generated a genome wide introgression series in the grasses, the largest introgession resource developed in the monocots. Highlights of this work include the demonstration of a 1:1 relationship between recombination and chiasma formation and the absence of chromatid interference across the centromere; the majority of genes in the monocots are located proximally in recombination poor regions; the isolation of the gene responsible for Mendels ***I*** locus [4]. This sequence is presently being used in breeding programmes and is an excellent example of basic research leading to the development of commercial varieties). King will be assisted in the alien introgression pillar by Julie King and Ian Armstead. Time commitment: 20%.

Theme leaders:

Keith Edwards and Gary Barker (High throughput genotyping). Keith Edwards and Gary Barker have made important advances in the field of marker development and genome organization/gene expression in cereals. This includes: the study of cold and light induced changes in the transcriptome of wheat in the transition from vegetative to reproductive growth (BMC Plant Biol. 2009 9: 55*)*; and the genome-wide analysis of single nucleotide polymorphism diversity in the world's major cereal crop [5] and multiplex SNP-based genotyping in allohexaploid wheat using padlock probes [6]. The group will use their existing Next Generation Sequencing (NGS) facilities in conjunction with existing SNPs and SNPs currently being developed in previously BBSRC-funded projects to genotype lines and populations generated by the partners. Time commitment: KJE 10% GB 10%.

Peter Shewry (Phenotyping). Peter Shewry has over 30 years experience studying the development, composition and end use properties of cereal grain and will evaluate the possibility of developing work on this topic during the course of the programme. He also has extensive experience in leading and managing complex multisite programmes: EU FP4 EUROWHEAT (€1.2m, 1996-1999), EU PF4 HEALTHGRAIN Module 2 (~€3m, 2005-2010) and BBSRC EX-Gen (9 partners, 2002-2007) and CSI (6 partners, 2006-2010) projects. He also chairs the Defra WGIN Management Group. Time commitment: 5%.

Additional members:

John Foulkes and **Erik Murchie** (Nottingham) will contribute to the programme on high-throughput screening of germplasm for input efficiency (N and P) and yield components. John has 17 years' experience in wheat physiology focusing on understanding the processes determining genetic variation in nitrogen-use efficiency and the developmental, genetic and physiological bases of ear fertility in wheat. He leads the Nottingham component of the Defra Wheat Genetic Improvement Network (WGIN) and is PI on BBSRC projects elucidating traits, mechanisms and genetic markers to improve nitrogen-use efficiency (BB/E527147/1) and raise ear fertility (BB/D008972/1) in wheat. Research on NUE has quantified N requirements of wheat canopies according to structural, photosynthetic and reserve functions of N and shown how NUE is related to canopy N dynamics and stay-green properties. Parallel work on ear fertility is investigating how novel genes introgressed into UK germplasm from CIMMYT wheats boost grain number through an extended duration for spike primordia production and optimised tillering. Erik has 12 years' experience in leaf photosynthesis in C3 species. His research focuses on factors that limit photosynthesis in crop plants, examining fundamental processes such as light harvesting, carbon

assimilation and energy dissipation. He is PI on a BBSRC project addressing genetic manipulation of photoprotection and photooxidative stress tolerance in rice (BB/G003157/1). The group will contribute to the phenotyping of parental donor and pre-breeding lines at Nottingham in replicated microplot trials under high and low N. Phenotypic screening of germplasm will include canopy size/architecture (spectral reflectance indices), photosynthetic efficiencies (Licor 6400 carbon assimilation), straw and grain yield (and N content) and yield components. Time commitment: John Foulkes (10%), Erik Murchie (5%).

Kim Hammond-Kosack is the Deputy Director of the Centre for Sustainable Pest and Disease Management at RRes. KHK group's research focus includes a disease of wheat ears caused by the Ascomycete fungal pathogens *Fusarium graminearum* and *F. culmorum*. The experimental approaches used have included functional genomics, genetics, biochemistry and bioinformatic analyses and have involved cereal and non-cereal host plant species and both fusarium species (BBS/B/12261). Many different wheat germplasms under both field and non-field conditions have been evaluated to identify lines that exhibit good resistance and low mycotoxin levels in the harvested grain. KHK has extended this approach to study Take-All with **Richard Gutteridge,** also at RRES. RG has more than 30 years experience of working on Take-All. KHK and RG are currently investigating sources of resistance to Take-All caused by the fungus *Gaeumannomyces graminis* var. *tritici*. in hexaploid wheat and in the wild diploid *Triticum monococcum*. More recently, in association with the Wheat Genetic Improvement Network (WGIN) project, KHK and RG have developed methods to screen for resistance to Take-All of different wheat genotypes in both pot tests and field plots, an approach which will be deployed within this proposed pre-breeding programme. Time commitment: KHK and RG 10%.

Malcolm Hawkesford leads the 'Input Traits (mineral nutrients, carbon and water)' group in the Crop Genetic Improvement Centre at Rothamsted Research. Work focuses on N and other minerals (S, P, Fe, Zn, Mo and Se) with emphasis on acquisition and transport processes, post anthesis canopy senescence and implications for yield and nutrient partitioning, including recycling mechanisms. Approaches include mapping of traits, analysis of natural variation in nutrient and particularly N-use efficiency parameters and in transcriptome studies. This work is/has been supported by funding from BBSRC competitive grants: BB/C514066/1 'An integrative transcriptome and metabolic profiling study of resource mobilization in wheat', BB/G022437/1 'Improving the N response of UK wheat varieties'; from DEFRA: AR0911 'Smart plant technology for sensing crop nutritional status' and AR0714 (a desk study) 'A study of the scope for the application of crop genomics and breeding to increase nitrogen economy within cereal and rapeseed based food chains', and IF0146, the Wheat Genetic Improvement Network; as well as industrial support, both independently and intrinsically involved in these projects. Recent work has pioneered a novel approach combining field scale agronomic experimentation with state-of-the-art 'omic technology, including utilising the long term field experiments at Rothamsted (BBSRC funded project 'Effect of nitrogen supply on the wheat endosperm transcriptome', Ref 206/D16781/2). The laboratory combines expertise at field scale assessment with laboratory-based gene analysis. The BBSRC project (BB/C514066/1) successfully collected Affymetrix and metabolite profiling (NMR) data from the field material. He manages several dedicated N-variety trials including the large WGIN N-variety and mapping population trials. Time commitment: 10%.

Martin Parry is the Head of the Department of Plant Science, and an internationally recognised expert on the regulation of photosynthetic carbon assimilation. He directs research on projects concerned with carbon assimilation and water use efficiency of wheat and willow. Recently this involved the creation and phenotypic characterisation in the field of a large mutagenised population (6000 lines) of Durum wheat from which mutations in candidate genes are being identified by TILLING. His current research is supported by BBSRC Institute Strategic Programme Grants (Crop Genetic Improvement (Co-Pi) and Bioenergy and Climate Change (Co-Pi)), BBSRC Crop Science Initiative (Enhancing wheat field performance and response to abiotic stress with novel growth-regulatory alleles (Co-Pi)), Defra (An integrated approach to increasing water use efficiency and drought tolerance of wheat production in UK (Co-Pi with Lancaster University) and the European Union (INCO-MPC OPTIWHEAT Improving the yield stability of Durum wheat under Mediterranean conditions). Time commitment: 5%.

John Pickett is Head of the Department of Biological Chemistry and Scientific Director of the Centre for Sustainable Pest and Disease Management. He is the world authority on semiochemicals in insect behaviour and plays a leading role in the move away from traditional wide-spectrum use of pesticides to more precise control through compounds targeted against specific pests at critical stages in their life cycles. Work centres on chemical ecology of interactions between insects and between insects and their plant or animal hosts. This specifically involves the chemical characterisation of molecular structures for semiochemicals that influence the development and behaviour of insects and some other organisms. Research extends to the biochemistry and molecular biology of secondary plant metabolites that act as semiochemicals the mechanisms by which they are employed by insects. More recently, his interests have turned to deriving ways of linking genomics through novel approaches to the metabolomes of insects and their hosts. He will lead the investigation of insect resistance within populations of wheat plants created by the LOLA and will be exploiting underpinning research on plants developing robust resistance to insect attack and colonisation, which involves a greater exploitation of primed and induced defence in crop plants. Time commitment: 10%.

John Snape has over 35 years experience of cereal genetics and biotechnology and currently leads a research programme investigating the genetic control of yield and yield components in wheat under high input and stress conditions (drought, low N) through large scale QTL and physiological analysis. The work includes the development and use of existing and new doubled haploid mapping populations and an extensive collection of new near-isogenic lines. Prof Snape has led the Defra-Funded WGIN project, is currently PI on four Crop Science Initiative projects, two Defra-BBSRC LINK projects, and PI on the BBSRC-INRA NUE project. Time commitment: 5%.

Cristobal Uauy was recently hired at the John Innes Centre (JIC) as a Project Leader for wheat gene cloning and is a Visiting Research Fellow at the National Institute of Agricultural Botany (NIAB) in Cambridge. His work has focused on the use of wild emmer (*Triticum dicoccoides*) to identify novel alleles for important agronomic traits. This led to the map-based cloning of the first QTL in polyploid wheat, *GPC-B1*, a transcription factor regulating senescence and with pleiotropic effects on grain mineral concentration [7]. He also identified *Yr36*, a resistance gene which confers partial and broad spectrum resistance to wheat yellow rust [8] and completed the positional cloning and final validation [*9*]. Functional alleles for both genes were absent from all modern wheat varieties and his work led to their re-introduction into the modern gene pool and their use by breeders. Uauy will be involved in the development of the wild emmer and *Ae. tauschii* introgression lines (ILs). He will also phenotype and develop new germplasm to capture diversity from *T. dicoccoides* for important traits such as canopy senescence and architecture, radiation use efficiency, grain filling duration and cuticular wax composition. These traits are characterized by their large environmental dependency and the multiple genes controlling them (known as quantitative trait loci (QTL)). Time commitment: 10%.

Competitiveness/Improvement quality of the proposer's research:
The research undertaken by the participants of this proposal has made a major contribution to wheat research nationally and internationally from the genetic dissection of yield potential, quality, adaption, biotic and abiotic stress tolerance to the identification of high protein and chromosome pairing genes, through to the synteny concept and SNP genome analyses. All the facilities required to successfully carry out such a task are available at IBERS, RRES, NIAB, JIC and the Universities of Bristol and Nottingham.

References
[1] Moore G., *et al.* (1993) Nature Biotech*.* **11**: 584-589; [2] Griffiths S., *et al* (2006) Nature **439:** 749-752; [3] Al-Kaff N., et al (2007) Ann Bot doi 10.1093/aob/mcm252; [4] Armstead I., *et al.* (2007) Science **315**: 73; [5] Imelfort M., *et al.* (2009) Plant Biotech*.* J. **7**: 312-317; [6] Edwards K.J., (2009) Plant Biotech. J*.* 7: 375-390; [7] Uauy C, *et al*. (2006) Science **314**:1298-301; [8] Uauy C., *et al.* (2005) Theoretical and Applied Genetics **112**: 97-105; [9] Fu D., *et al.* (2009) Science **323**:1357-1360

Part 1B Statement on Data sharing

We strongly support free and unrestricted access to publicly funded research. In the past we have taken steps to make our results easily and freely accessible. For example, all the sequence data generated have been deposited in the appropriate public databases. In some cases in our previous research there have been no suitable public databases and in these cases we have provided accessible suitable databases hosted by our institutes. In this project we will archive all data in accordance with BBSRC guidelines.

Data areas and data types: Raw data, generated by Illumina GA2 Next Generation Sequencing (NGS), will be stored temporarily to extract sequence and quality scores then deleted. The sequence files will be aligned and used to identify sequence polymorphisms for subsequent genotyping.

Relational data and Metadatabases: The programme will generate tens of millions of genotyping/phenotyping datapoints together with the associated metadata. To make full use of this data within the pillars, maximise synergies across the programme and make the data fully accessible, JIC and Bristol will work together, using as a base their current database development work, to establish a "fit for purpose" relational database. As such, our data will be a vital community resource and it will provide a key foundation for numerous follow on projects.

Secondary use: The sequence/genotyping data will be used by the research community and the wheat breeding industry to implement, on a large scale, molecular breeding methods to improve the wheat crop worldwide.

Methods for data sharing: Sequence/genotype data and assemblies will be submitted to EBI depositories as soon as practicable after generation. The Bristol-based BBR programme will provide the primary interface to breeders and the global wheat research community, and will be linked with the Monogram network project and comparative databases at JIC and the rest of the world via the wheat SNP consortium. All the mapping and phenotyping data will be available on websites developed at University of Bristol and QTL data on websites at JIC. Seed will be stored in dedicated short, medium, and long term gene bank facilities at JIC. Accession numbers and passport information will accessible, as they are now, for all accessions held in the BBSRC small grain cereals collection.

Publications: In principal we support publication in Open access journals. However we also recognise that currently post-docs and students need recognition that publication in established high impact, non-open access journals provides for their career progression. Therefore, in the present project, we will ensure that all published papers are freely accessible, either through the publishers (as in the case for several journals that we publish in), or by submitting independent copies to Pubmed Central or similar open access sites, for other journals that we publish in. We will also make our publications available on our web pages.

As part of the post-doctoral training on this grant the post-doctoral fellows will be expected to present their work both internally and externally at open meetings. The PIs will also present the work at open scientific meetings.

Sharing data with the wider community: The results of the work will be communicated to the general public, where appropriate, through press communications and made through newsletters issued through the participating organisations. The outputs of this research are likely to be of benefit to plant breeders at present and into the future, possibly in the Developing World. We therefore feel it is important that these outputs are not encumbered by any IP restrictions. All the germplasm developed will be available free of IP restrictions to public sector researchers and private sector breeders but covered by appropriate MTAs.

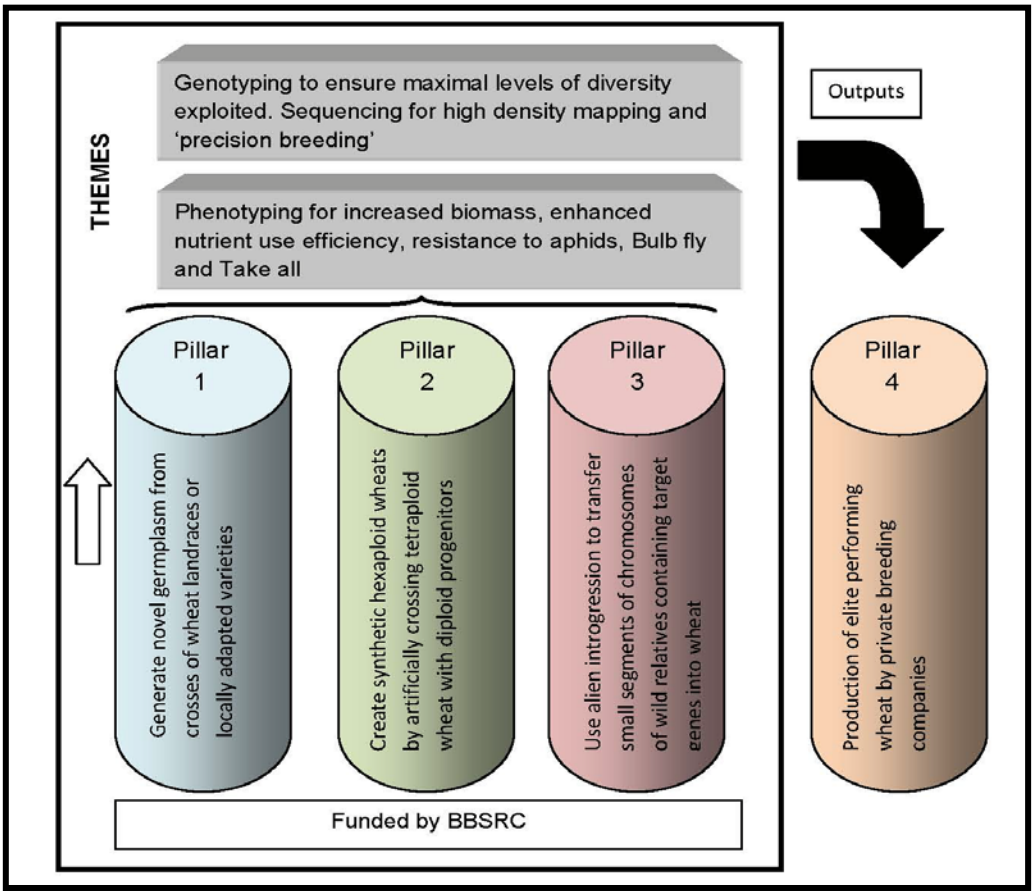
Each of the centres will hold open days so that researchers and breeders outside the programme can come and view the material developed each year.

Part 2 Description of proposed research

The problem: Food security is becoming a critical issue both in the UK and worldwide due to rapid population expansion, dietary changes and declining stocks of fossil fuels. Total wheat grain production over the next 50 years must exceed that previously produced over the last 10,000 years, since agriculture began. The UK's current food and farming ecological footprint is up to six times the food growing area of the UK. It is no longer feasible for the UK to rely on wealth created from its service sector to buy a decreasing supply of grain on the open market. The US are planning to double maize yields by 2030 using 30% less land, water and energy through the deployment of biotechnology. The UK needs a similar vision for food production covering its major crops, such as wheat, to address the potential market failure created by the fact that commercial plant breeding generates insufficient returns to justify the level of investment which addressing these critical targets will require.

Solution: Preventing this market failure will require a unified approach by academic institutions to develop a wheat pre-breeding programme to enhance and underpin the successful UK private breeding sector. This need is endorsed in the second key recommendation by the Royal Society's report "Reaping the benefits" 2009 on addressing Food security which recommended funding for the UK public sector to undertake pre-breeding in wheat. The objective of the proposed programme is the development of pre-breeding germplasm, characterised for key traits, and the identification of genic markers for selecting these traits, for use both in commercial breeding programmes and for academic research.

Figure 1: Structure of the programme



There will be **three public pre-breeding sub-programmes or "pillars**", each of which will broaden the pool of genetic variation in wheat by a different route (Figure 1). These are:

- The **Landrace pillar** which will develop germplasm from crosses involving wheat landraces (locally adapted varieties), derived from the Watkins and other collections.
- The **Synthetics pillar** which will create synthetic hexaploid wheat by artificially crossing tetraploid wheat with diploid wheat progenitors. This captures diversity in both the tetraploid and diploid wheat progenitors. The potential impact of these synthetics is illustrated by their successful exploitation within the CIMMYT wheat breeding programme.
- The **Alien pillar** which will use alien introgression technologies to transfer small segments of chromosomes of wild and cultivated relatives containing the target genes, into wheat. Wild and cultivated relatives (alien species) provide a wealth of genetic variation for all characters of importance relative to yield, climate change and the environment.
- A **fourth pillar,** which will involve the production of elite wheat cultivars, will be resourced independently by the private breeding companies.

Two cross-linking themes, genotyping and phenotyping, provide the "Entablature" connecting the "Pillars". The parental material used in the initial pre-breeding crosses will be genotyped ensuring the maximal levels of diversity are being exploited. Second and third generation sequencing will generate very high density maps, providing the breeding companies with markers for "precision" breeding, and academia with markers for fine dissection of key traits. The participation of a large number of current researchers, with specific trait expertise in the programme, means that the germplasm produced from the three pre-breeding pillars will be screened for plant architecture and phenology, productivity traits, pest and pathogen resistance and input use efficiency. The resulting germplasm will be exploited by breeders for crossing with their elite lines, and used by academics to study the biology of new traits.

Strategic relevance of the project to BBSRC: BBSRC research priorities reflect the growing awareness of food security as a future problem [1]. There are whole research priorities dedicated to Global security and to Crop science. BBSRC states that this will be achieved "by implementing the strategy and recommendations outlined in its Crop Science Review and by building on BBSRC's earlier investments through response mode- grants and core funding of institutes and managed initiatives". Recommendation 14 of the Crop Science Review, endorsed by Council, stated that "BBSRC should take the lead to establish a national plant breeding initiative that would promote public good breeding by establishing crop genetic improvement programmes with the aim of providing improved germplasm and technology for the development of new varieties". Recommendation 17 stated that "BBSRC should seek to increase publicity for public good plant breeding". The common objective present in all these BBSRC priorities, recommendations and impact plans is the establishment of pre-breeding for the UK's major crops, such as wheat, an imperative that has yet to be implemented.

The present proposal therefore seeks to address the requirements of the BBSRC's impact plan by re-establishing a publicly funded wheat pre-breeding programme. Wheat is the UK's largest crop with an annual production of 14 million tonnes and market values for its seed and processed products of around £1.4 billion and £14 billion, respectively. Wheat yields are not increasing at comparable rates to those achieved in previous decades. Diversity has been eroded during its domestication and is being further eroded during selection. We need to halt this trend and enhance the diversity of wheat through exploiting exotic sources in breeding programmes. This programme will re-establish the exploitation of experimental crosses involving wild wheat, landraces, synthetic bread wheat and grasses (pre-breeding germplasm) in the UK, as a means of transferring traits of high agronomic potential. These lines will be a new source of diversity but will require further breeder selection to generate varieties with elite performance for release. The resulting germplasm has the potential to possess resistance to a range of pathogens and insects, tolerance to low N, drought, salt and heat as well as exhibiting enhanced yield characters. This integrated programme will address a broad range of BBSRC research priorities as stated above. It will also provide a pipeline for the translation of fundamental science into applied outcomes for exploitation by the UK breeding industry.

Strategic relevance of the project to the goals of the host institutions. Institutes and Universities need to train the next generation of researchers in a range of skills capable of

addressing the issues surrounding Food Security on behalf of the UK. After privatisation of the PBI, wheat research in the public sector became dispersed across many different institutions. Future researchers need to have exposure to this entire skill base. The proposed wheat pre-breeding programme involves researchers from six different types of UK institutions, NIAB, IBERS, RRES, JIC and the Universities of Bristol and Nottingham which operate separately from each other in the field of wheat research but have combined skills in genomics, genetics, biochemistry, breeding, and physiology of wheat. No single institution has the skill base to develop a wheat pre-breeding programme in which germplasm from landraces, synthetics and alien introgression is genotyped and phenotyped. This programme brings together this unique range of skills, bringing added-value through exploiting the synergies and complementarities between the partners. By each institution contributing to a part of the programme, it enables that institution to impact on Food Security in a way it could not by operating separately. Moreover, it provides each institution with access to a range of skills equipping the next generation of researchers in the skill base required to address Food Security in the coming years.

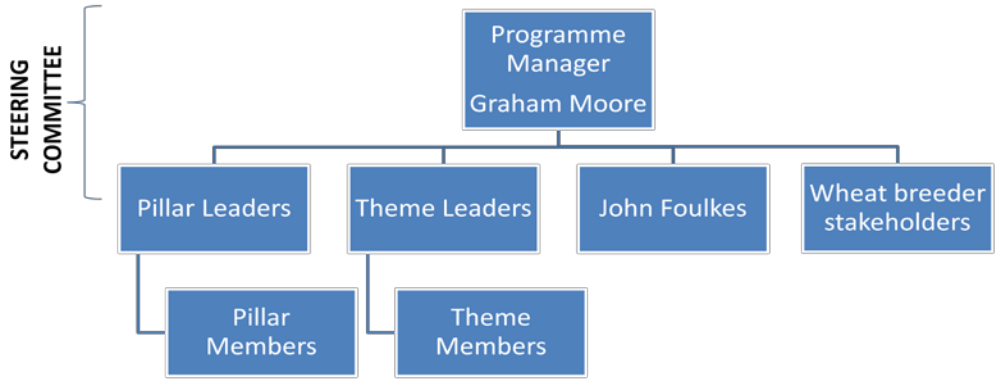
Training: Much of the current expertise in UK wheat genetics exists due to past investment in training from sources that no longer exist. The entire programme 'Enhancing diversity in UK wheat through a public sector pre-breeding programme' will make a long term contribution to attracting the best young scientists into wheat genetics from application in breeding programmes to elucidation of underlying mechanisms. The programme will provide the basis for new projects and act as catalyst for growth in the UK wheat research community. In addition to these activities, the Landrace pillar will establish an undergraduate summer school organised through the JIC summer programme [2], exploiting recent investment in training facilities at JIC. The course will cover all of the elements of this research pillar giving students a unique insight into wheat genetics from field to gene. At the postgraduate level related projects will be offered for MSc and PhD.

Programme management. This is a large and ambitious programme and will require coordination and involvement of the key stakeholders, the breeding companies. The project therefore has a formal management structure and a steering committee. Graham Moore will be Programme Manager (PM). To remove any conflicts of interest the PM's research group will not be funded, although his research is being exploited through this programme. If this proposal is funded, the PM will step down from his other management responsibilities, so that he can devote 20% of his time to overseeing the success of the project. The costs of the time that the PM spends managing the programme are included in the grant. The PM is highly committed to ensure there is a framework for translation of academically excellent research. The PM's role will include, the day to day management of the programme, dealing with reports, facilitating interactions and ensuring that staff are available from within the programme to deal with activities which require a large number of "hands" at specific times of the year. He will facilitate interactions with the breeders and when necessary act as an arbitrator between the demands of the academics and those of the breeding industry.

A researcher will be responsible for each of the pillars and the two cross-themes (Simon Griffiths-Landraces, Andy Greenland-Synthetics, Ian King-Aliens, Peter Shewry-Phenotyping and Keith Edwards-Genotyping-bioinformatics) and will serve on the steering committee. John Foulkes representing Nottingham University is also a member. The steering committee consists also of representatives from the wheat breeding Industry, Bill Angus (Limagrain UK), Simon Berry (Limagrain UK) , Chris Tapsell (KWS-UK), Peter Werner (KWS-UK), Richard Summers (RAGT), Peter Jack (RAGT), David Feuerhelm (Syngenta), David Thompson (Syngenta) and Stephen Smith (Elsoms). A representative from the CIMMYT wheat breeding programme will also be a member of the steering group. John Snape will act as an advisor to the steering group. This committee has met to agree the structure of the programme, populations, genotyping and phenotyping targets. During the course of the programme, this committee will meet formally twice a year to review the progress within the programme at one of the centres in the programme. The academic representatives (Andy Greenland, Keith Edwards, Ian King, Peter Shewry, Simon Griffiths, John Foulkes and Graham Moore) of the committee will have a formal discussion on progress within the programme every month via a video conference. Each of the centres involved

in the proposal will also organise an open day each year for the breeders to come and assess the germplasm being grown.

Management structure of the pre-breeding programme



Industrial contribution. As described above, the UK has largely stopped producing experimental crosses with wild wheat and grasses because the wheat breeding industry has insufficient funding to sustain this activity. This LOLA will support the activity through public sector funding. Much of industrial contribution and commitment to this programme will come in subsequent years with assessment of the value of the promising germplasm developed within the programme, including validating for useful traits and determining whether favourable alleles for one trait carry undesirable consequences for others. The breeders have been, and will be, actively involved in giving their advice on germplasm development genotyping and phenotyping, and being involved in the management steering committee.

Pre-breeding Pillars and cross-linking themes

Pre-breeding Pillar 1: Exotic Bread Wheat-Landraces

Overall aim. To develop germplasm which facilitates the identification, dissection, and assessment of novel genetic variation for traits that are available in landraces and other exotic bread wheat lines, but not yet deployed in current UK germplasm, and facilitate the deployment of this variation into UK wheat breeding programmes.

Background: The genomes of modern, elite bread wheat varieties are mosaics of chromosomal segments originating from wheat lineages (land races) that arose during the 10,000 years separating the domestication of hexaploid wheat and the birth of modern plant breeding. As plant breeding progressed through the 20th century, quantitative genetic gain was increasingly achieved through the deployment of already selected alleles into new combinations. This is a very efficient approach for the fine tuning of germplasm for a specific set of environments. However, the search for new and useful allelic variation outside of adapted germplasm is challenging for commercial wheat breeders. This is an essential element of any strategy for continuance and consolidation of the genetic gains achieved for UK wheat, as well as an opportunity to arm UK wheat breeders with alleles that will facilitate adaptation to *new* environmental challenges: reduced nitrogen inputs; drought; thermal stress; and new pests and diseases. The work proposed here will provide greater resilience and flexibility for the existing academic to commercial genetic pipeline for UK wheat breeding.

Physiological, agronomic, and breeder's models of mechanisms underlying target traits will guide extensive phenotypic screens of landrace and non-adapted germplasm collections. This is a scoping exercise for the extent of variation available. It will guide the selection of parents for the development of segregating populations in which the genes controlling traits of interest will be characterised as quantitative trait loci (QTL). Potentially useful alleles will be introgressed into a set

of elite genetic backgrounds so that their value for breeding can be assessed against the best existing varieties. This work will deliver pre-breeding germplasm for UK varietal improvement in Pillar 4 and also to world wheat breeding. It will test and guide modification of the physiological models driving this programme. The genetic materials produced, together with associated phenotyping and genotyping inputs of parallel work packages, will facilitate the positional cloning and elucidation of molecular genetic mechanisms underlying these key traits via 'uplift' activities. The ultimate outcome of this work, together with the UK existing wheat genetics and physiology programme, will be sustainable genomics led predictive wheat breeding in the UK.

Research Programme:

Sources of new genetic variation in hexaploid germplasm: The UK wheat genetics community has made a long term investment into four germplasm collections that are highly suited to the objectives and scale of the work proposed here. **1)** The AE Watkins Collection comprises 831 land race accessions collected from thirty three countries in the 1920s and 1930s. The collection of Watkins samples, prior to the introduction of improved varieties in these countries eliminates the possibility of contamination from modern wheat varieties and provides a genuine snapshot of global wheat germplasm prior to systematic inter-crossing and selection. As part of the Defra funded Wheat Genetic Improvement Network (WGIN) each accession has been genetically fixed and multiplied (enough seed to accommodate the needs of the proposed work immediately). Moreover, data for a number of traits including height, heading date, grain shape, glutenin composition, foliar disease, growth habit, and morphology have been collected. Over twenty single seed descent (SSD) populations are under development and will be available. **2)** The Gediflux Collection was assembled as a JIC contribution to an EU FP6 project. It comprises over 500 Western European winter wheat varieties that individually have occupied over 5% of National acreage from 1940 onwards. **3)** A collection of lines with extreme phenotypes largely collected by John Snape and the late Tony Worland at JIC. **4)** Non UK parents of existing mapping populations that can be used for the identification of QTL. An example of this type of material would be the key CIMMYT varieties Weebil, Bacanora, Milan and Catbird. JIC has already produced doubled haploid (DH) populations and genetic maps for these varieties and identified QTLs for a wide range of traits in diverse environments.

As the programme progresses new sources of genetic diversity will be identified and multiplied for characterisation beyond the three years of research proposed here.

Primary germplasm screens: Within the Landrace pillar, we will maintain seed stocks and supply seed and tissue aliquots to project partners. We will also continue to collect data for agronomic traits including crop height, heading date, establishment, and grain yield components. Project partners leading the phenotyping cross linking theme (Rothamsted and Nottingham) will undertake detailed physiological sampling and analysis at this stage. This analysis is described in more detail in the Phenotyping Cross linking theme work plan, but an example would include multi site measurement of component traits for NUE, grain yield, phenology, and biomass of 100 AE Watkins lines in each year of the programme. These screens will inform the choice of up to 25 parents for the production of segregating populations or introgression of target alleles into elite variety backgrounds from year 2 onwards.

Genetic dissection: None of the segregating mapping populations initiated during the project will be available for phenotypic screening, in sufficient quantity, during the proposed funding period of three years. However, this work is part of an ongoing programme and over fifteen non UK adapted populations, including ten Watkins x Paragon SSD are available for immediate use. We will be responsible for multiplying and distributing them. The gene discovery programme of the Landrace pillar will employ well proven methodologies to increase the capacity of the pipeline that produced the existing populations. Choice of parents will be informed by the Phenotyping cross matching theme. Biparental crosses will feed SSD and DH population development. In the majority of cases the UK parent will be the elite spring wheat Paragon. Non adapted parents will be genotyped for genes controlling response to photoperiod (*Ppd*) and vernalisation (*Vrn*). Photoperiod insensitive and winter genotypes will then be removed by marker assisted selection (MAS) at F_2, prior to full population development. This will reduce the heading date window for UK field conditions, greatly

increasing the precision of physiological measurements. Maximum SSD capacity at any one time will be 5000 lines. Maximum DH capacity will be 300 lines per year. The high throughput parallel marker platforms developed under the Genotyping cross linking theme will complement existing platforms (DArT, SSR, and COS) in the production of 300-500 loci genetic maps. Initially bulked segregant analysis (BSA) will be used to efficiently assign segregating alleles to QTL. Full population screens will confirm these locations and QTL of interest will be selected for the next module. QTL data will be archived in a publicly accessible relational database (see Data handling below) and placed onto a wheat consensus map.

Assessing the impact of new alleles in elite germplasm: We will identify different QTL categories in terms of the effect of the landrace allele relative to the Paragon allele; 1) Superior performance, 2) neutral effects 3) deleterious effects. Priority will be placed on effects in that order, but even category 3 effects are of interest. The purpose of this programme is to not only identify genes for *existing* UK farming environments, and several examples exist of allelic effects which are beneficial in one environment, but deleterious in another. Up to 20 QTLs a year will be selected for backcrossing into Paragon and a subset into a panel of elite wheat varieties representing six CIMMYT defined mega environments (ME1,4,6,8,11, and 12) of strategic significance for UK research, breeding, and farming. Backcrossing into a range of genetic backgrounds, and the use of these materials by commercial wheat breeders, will require the development of diagnostic molecular markers. Markers polymorphic in the population used for gene discovery will not necessarily be useful in other crosses. To address this issue, we will interact with the Genotyping cross linking theme and commercial plant breeders to screen the seven recurrent parents with all markers available for regions of interest. Informative markers will be used for MAS during the backcrossing programme. NILs will be screened in multiple environments where they will be assessed for expression of the trait of interest (Phenotyping cross linking theme) and agronomic performance (Landrace pillar with commercial breeding pillar). In cases where NILs are agreed to have potential benefit in breeding programmes, the varieties comprising the UK winter wheat recommended list (as of 2010), will be screened with the same marker set used to screen NILs, in order to develop molecular markers compatible with commercial breeding high-throughput platforms.

Physiological hypothesis testing: The primary germplasm screens and prioritisation of QTL will be based on physiological and agronomic models defined in the Phenotyping cross matching theme. These models will address how traits (phenology, photosynthetic capacity, partitioning, nutrient use efficiency etc) interact to determine crop performance. Genetic adaptation of each process will benefit from estimation of likely interactions and interdependencies between processes. In addition to QTL validation and pre-breeding material the NILs produced by the Landrace pillar provide the essential raw materials to test these models. For example, if changes in phenology predicted to positively influence grain yield potential are borne out, then the applicability of that model to targeted environments is supported and the programme will prioritise efforts in that area. Alternatively, if a push towards another phenotype does not deliver, then emphasis will shift away from these areas. In the longer term (beyond three years) NILs can be inter-crossed to assess the interaction between traits as some alleles might need to be selected with specific combinations of interacting alleles. This work will allow physiologists, agronomists, and breeders to modify models and ideotypes and will inform MAS strategies.

Data handling: Within the Landrace pillar, we will generate data for up to 2,000 landraces/unadapted varieties and 75 segregating populations. This is likely to include approximately 4,000,000 genotype data points, 2,000,000 phenotype data points, weather records and field records for all sites and years. It is difficult to estimate the number of QTL that might be identified, the NILs developed for each QTL, records of available seed, and tracking of requests for that grain. To make full use of this data within the pillar, maximise synergies with the rest of the programme, and make data fully publicly accessible, it is essential that a relational database is developed within the pillar. This will build on current database development work currently underway on a collaborative basis including ISP investment at JIC, Germinate, Crop Store, BBR, Monogram, WGIN, and ISIS.

Summary of Outputs:

1. Multiplication and curation of exotic bread wheat germplasm collections for use across the programme.
2. Contribution to primary phenotypic screens of germplasm collections.
3. Mining of novel alleles for genes of known function.
4. Production of segregating populations (25 each year) for lines of interest identified in primary germplasm screens.
5. Use existing landrace x elite segregating populations to identify QTL controlling traits of interest
6. Production of Near Isogenic Lines for prioritised QTL and genotypically defined allelic variants.
7. Assessment of agronomic performance of Near Isogenic Lines.
8. Development of informative genetic markers for marker assisted selection in commercial wheat breeding programmes.
9. A relational database for full public access to data generated.

Pre-breeding Pillar 2 Inter-Specific (Synthetics) Crossing

Overall Aim. To extend the bread wheat gene pool by understanding, exploiting and incorporating novel genetic diversity from diploid and tetraploid Triticeae genomes. The principal objectives are to **1)** create backcross lines in adapted germplasm for breeder exploitation using two inter-specific crossing approaches (synthetic hexaploid wheat and hexaploid x tetraploid) **2)** develop three wild emmer bi-parental mapping populations as resources for trait dissection and **3)** identify founder lines for the future development of chromosome segment substitution lines (CSSL) for QTL mapping and positional cloning.

Background. A large degree of genetic diversity within a very accessible gene pool is available from diploid goat grass (*Aegilops tauschii*) incorporated in synthetic hexaploid wheat (SHW) and from tetraploid sources such as wild and cultivated emmer wheat (*Triticum dicoccoides T. dicoccum*) and durum wheat (*T. turgidum*)) used as direct donors in crosses with bread wheat. SHW re-create the original hybridization event that occurred between durum wheat (*T. turgidum*; 2n=4x=28 AABB) and *Ae. tauschii* (2n=2x=14 DD) some 10,000 years ago. This approach has been widely used to broaden genetic diversity in bread wheat where there is comparatively little D-genome variation; recent estimates indicate that more than one third of CIMMYT's advanced breeding lines are SHW derivatives [3].

To demonstrate the UK potential of SHW BBSRC has funded NIAB (BB/E006868/1) to develop backcross lines in adapted wheat genotypes using CIMMYT SHW; in 2009 the NIAB pre-breeding team interrogated an early-generation field nursery in conjunction with commercial breeders. This will be repeated with around 3000 advanced lines in 2010 and a smaller set of interesting lines will be progressed further. In parallel, to extend the use of SHW, the NIAB Trust has supported a 12 month pump-priming project to establish methods for creation of novel SHW, thus allowing exploitation of previously unused D-genomes. To date 24 new SHW have been produced from about 70 crosses. These two areas of expertise now embedded at NIAB will be fully exploited in this new programme.

1. Generation of synthetic hexaploid wheat lines. We will adopt two inter-specific crossing strategies to add genetic variation to UK bread wheat. Firstly, we will use synthetic hexaploid wheat (SHW) to introduce extensive D genome variation. Secondly, we will make direct crosses between two heaxaploid wheats and a range of diverse tetraploid donors (Hexaploid x Tetraploid Wheat – HxTW). This introduces novel variation in the A and B genomes and saves time, relative to SHW, as it avoids embryo rescue and backcrossing can start as soon as the pentaploid F_1 is identified.

We will use Paragon and Robigus as backcross parents for SHW and HxTW. The former is a spring-sown wheat without a vernalization requirement, thus allowing 3 generations per year. In contrast only 2 generations are possible with Robigus as it is an autumn-sown "winter" wheat,

representative of the majority of wheat sown in the UK, and needs 6 weeks' cold treatment for vernalisation. Critically, both varieties work well as parents in our current programme, displaying little hybrid necrosis when crossed with CIMMYT SHWs. In both strands, SNP genotyping will be used to characterize novel genomic blocks transferred into the recipient genotypes and BC_1F_2-F_5 lines prepared for phenotyping and as donor materials for exploitation by commercial breeders.

1.1 Selection of Diploid and Tetraploid Donor Lines: The majority of the crossing work will focus on the breadth of diversity incorporated into adapted germplasm, so genotype data from SNP analysis and any known phenotype information will be used to select the bulk of diploid and tetraploid donor lines. However, as described below, phenotypic data will be generated to aid selection of wild emmer lines.

To make new SHW a collection of 360 diverse *Ae. tauschii* accessions assembled at NIAB will be used to select genotypes divergent from those used to generate the CIMMYT SHW. The latter accessions are predominantly from Afghanistan, Iran and Pakistan; we will concentrate on accessions from countries in the northern and eastern range of this species' natural habitats in the Middle East (Turkmenistan, Uzbekistan, Kyrgyzstan). As far as we are aware these accessions have not been extensively exploited in bread wheat breeding (pers. comm. J. Raupp). Around 50 *Ae. tauschii* donors will be selected on genotype to create synthetic wheat by hybridization with two durum lines that are good combining parents (e.g. Hoh-501-P14 and Hoh-501-P4-2 used in our current work).

In HxTW crosses, 25 cultivated emmer (*T. dicoccum*) and durum (*T. turgidum*) lines, including commercial elite durums nominated by breeders will be selected on genotype and known phenotype (e.g. disease resistance) from a group of 450 accessions. A different approach will be adopted for wild emmer (*T. dicoccoides*) as these genotypes are proven sources of interesting traits [4,5] and make a significant contribution to current high yielding bread wheat varieties such as Oakley. Around 25 wild emmer lines will be selected from NIAB's collection of 106 accessions on genotype and information on key traits such as canopy architecture, senescence, grain filling duration, radiation use efficiency and cuticular wax composition, all of which are expected to affect yield according to current physiological and agronomic models [6]. Thus 50 tetraploid donors will be advanced for production of HXTW.

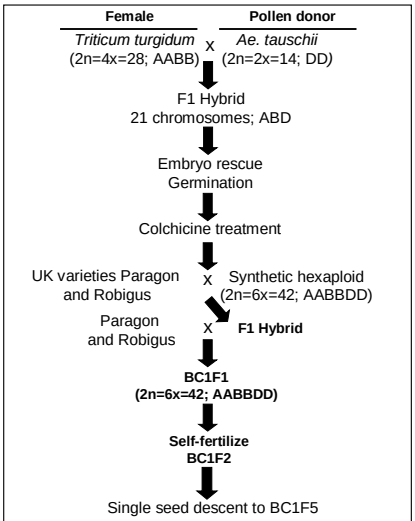


Figure 2.1. Synthetic Hexaploid Wheat Crossing Strategy

1.2 Generation of New SHW and Crossing with Hexaploid Bread Wheat: To generate new synthetic wheat material each of the 40-45 diverse *Ae. tauschii* lines will be crossed on to both durum parents described above and new SHW derived as described in Figure 2.1. In our current pre-project work 40% of crosses produce F_1 hybrid seed. We estimate that it will take up to 4 rounds over years 1 and 2 to complete the crossing work. This allows for crosses which fail to be repeated in a subsequent round. At appropriate points leaf samples will be taken for flow cytometry to check that the F1 hybrid plants are triploid using diploid and tetraploid checks and any tetraploid plants arising from self pollination or parthenogenesis discarded. Confirmed hexaploid SHW will be crossed to Robigus and Paragon to produce the F_1, backcrossed to the same recurrent parent and then allowed to self-fertilize to produce the BC_1F_2 (Figure 2.1).

From the NIAB Trust pump-priming pre-project described above, to establish the SHW methodology, we will select 5 of the 24 new SHW that meet this project's criteria for diversity. Backcrossing these SHW to Paragon and Robigus will be initiated at the start of the project. We expect the BC_1F_2 produced to be planted in the spring and autumn of year 2 (see "Field Plots" below).

1. 3 Crossing Hexaploid Bread Wheat with Tetraploid Emmer and Durum Donor Lines: Working from the selected set of 25 cultivated emmer and durum types and 25 wild emmer genotypes individual lines will be crossed to Robigus and Paragon to produce the F_1 (Figure 2). F_1 lines arising from HxTW crosses will be confirmed as pentaploids by flow cytometry against tetraploid and hexaploid checks and by chromosome counts from root tip squashes. Confirmed pentaploid F_1 lines will be backcrossed with either Paragon or Robigus to give the BC_1F_1. Plants in this generation can exhibit a range of ploidy types or be aneuploid (J. Snape, pers. comm); chromosome counts from root tip squashes will be used to select plants that are hexaploid and these plants allowed to self-fertilize to produce the BC_1F_2 (Figure 2.2).

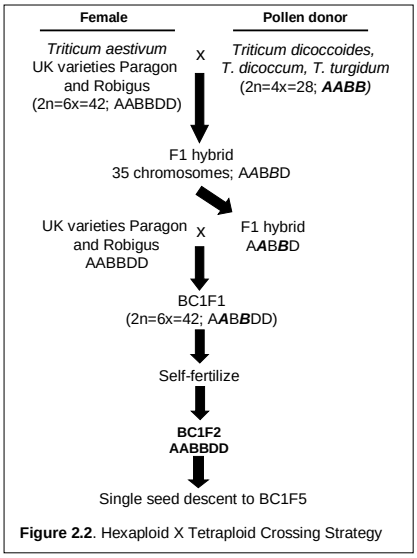


Figure 2.2. Hexaploid X Tetraploid Crossing Strategy

1.4 Field Plots and Line Progression*.* Small observation plots from the Paragon BC_1F_2 of HxTW derived from cultivated emmer and durum lines and SHW from the pre-project set will be planted in the field (Feb-Mar in year 2, assuming a project start date in Q4 2010) to assess variation in the families, discard some of the lines and identify up to 100 BC_1F_2 lines from each backcross lineage that look promising as future trait donors. Due to vernalisation Robigus BC_1F_2 lines will be around 4 months behind the Paragon BC_1F_2; this delay will allow us to ensure that any Paragon crosses that fail are attempted in the Robigus set. The Robigus BC_1F_2 will be autumn sown in year 2 and assessed in the field in year 3. HxTW derived from wild emmer and from early SHW should provide BC_1F_2 lines in Paragon that can be sown in as field plots in the spring of year 3. Crosses to produce BC_1F_2 of wild emmer HxTW in Robigus and later SHW in both Paragon and Robigus will be initiated but are unlikely to be available for field planting within the three year funding period.

From each of the 25 BC_1F_2 lineages derived from cultivated emmer and durum TxHW in Paragon and Robigus up to 100 inbred BC_1F_5 families will be produced by single seed descent. We expect to lose some lines due to sterility and lethality; a conservative estimate would be that 80% survive resulting in a final collection of 2,000-2,500 inbred lines from each of the 2 donor/backcross parent combinations. This process will be completed by the end of year 3. In the SHW stream only lines in Paragon (around 500) derived from pre-project SHW will reach BC_1F_5.

2. Development of wild emmer mapping populations: Wild emmer lines with contrasting phenotypes affecting yield characters will be crossed and three mapping populations developed from the F_2 generation by single seed descent. These populations are necessary to support the dissection of the genetic architecture of key traits described above by the wider community in future projects**.**

3. Development of single chromosome founder lines. Our target for production of D-genome CSSL will be a SHW developed from *Ae. tauschii* JIC-2220007 since we have an existing 8X BAC library for this accession. An appropriate AB-genome donor for CSSL will be identified in the phenotypic screen of wild emmer wheat lines in year 1 and a BC_1 population will be developed using Paragon as the recipient. Both CSSL populations will be screened with polymorphic markers to identify BC_1 individuals which carry the majority of their AB- or D-genome complement from Paragon, but which carry a single homozygous chromosome (intact, or with a single crossover). In subsequent work, beyond the scope of this funding period, these selected BC_1 individuals will be used as founders for further backcrossing in order to develop an ordered set of overlapping lines carrying incremental segments from the AB- or D-genome donor in a similar approach to that used for precision mapping of QTL in tomato and rice [7,8]. We anticipate that suitable lines will be recovered from the BC_4F_2 generation, although lines extracted from earlier generations may be used for preliminary QTL mapping.

Summary of Outputs:

1. Selection of diverse accessions of diploid and tetraploid donor plants (year 1).
2. Production of 50 pentaploid F_1 HxTW lines and 50 novel SHW (years 1-2).

3. In Paragon and Robigus produce 2000-2500 BC_1F_5 lines from 25 cultivated emmer and durum donors and 400-500 BC_1F_5 lines from pre-project SHW; initiate development of equivalent material from 25 wild emmer and 50 novel synthetic wheat (years1-3).
4. Physiological data on phenotypes expected to affect yield in emmer wheat; select genotypes for mapping population and CSSL production (year 1).
5. Initiated development of 3 wild emmer mapping populations (years 2-3)
6. Single chromosome founder lines from *Ae. tauschii* and emmer wheat for future production of CSSL populations as resources for QTL mapping and positional cloning (years 2-3)

Pre-breeding Pillar 3- Alien introgression programme

Overall aim: To significantly increase the gene pool of wheat via the introgression of genetic variation from related wild and cultivated *Triticeae* (alien) species.

Background: The wild relatives (alien species) of wheat provide a vast and largely untapped reservoir of genetic variation (for traits such as tolerance to abiotic and biotic stress, biomass, yield and photosynthetic potential). This variation can be exploited for the development of new high yielding varieties adapted to climate change and environmentally friendly agricultural practises. A few wild relatives of wheat have been successfully exploited as a novel source of genetic variation for traits in wheat breeding programmes, e.g. *Aegilops umbellulata*-leaf rust resistance [9,10]; rye-yield advantage and several genes for disease resistance [11].

Although wheat/alien introgression will enable us to meet many of the production and environmental challenges facing present and future wheat production its use to date, considering its promise, remains relatively limited. This was a direct result of a lack of adequate genetic markers and suitable technology platforms to identify and characterize introgressed alien chromosome segments. In addition, the majority of introgressions made involved large alien chromosome segments carrying deleterious genes as well as the target gene. Sears [9,10] developed a successful strategy to isolate smaller alien chromosome segments only carrying the target gene. This strategy involved the inter-crossing of two lines with different but overlapping alien chromosome segments but which carry the same target gene (Figure 4). As a result of recombination between the two overlapping alien segments, in the presence of pairing control genes, some of the progeny produced are recombinant, carrying reduced alien chromosome segments with the target gene, but lacking deleterious genes.

Research programme: We will exploit new marker technology, e.g. SNPs, comparative mapping, the sequencing of model genomes and Next Generation Sequencing (NGS) technology platforms to unlock the genetic variation within alien species. This will enable the generation of novel germplasm that will be exploited in the development of superior, adapted wheat varieties. In addition we will exploit our previous experience and tools developed in the grass/alien introgression system – the largest introgression programme undertaken within the monocots. Our *primary objective* will be to transfer small alien chromosome segments, carrying target genes but lacking deleterious genes, into wheat quickly and efficiently. The strategy that we will employ will be as described by Sears, i.e. the identification and inter-crossing of lines that carry different and overlapping chromosome segments followed by the analysis of the resulting progeny to isolate small introgressions. We will do this in two ways: 1) transfer of the entire genome of alien species into wheat in overlapping chromosome segments and 2) targeted development of overlapping chromosome segments for specific regions of the genome carrying target genes.

1. Whole genome introgression of alien species into wheat in overlapping segments: Four diploid (2n=2x=14) species have been selected for this work: *T. urartu* (wheat A genome donor, implicated in photosynthetic capacity and disease resistance etc), *Thinopyrum bessarabicum* (highly salt tolerant, potential donor of genes for heat tolerance, drought and disease resistance), rye (a specific genotype which has resistance to all known rust diseases, heat tolerant, drought tolerant, resistance to acid soils etc) and *Ae. speltoides* (wheat B-genome donor, disease resistance, potentially insect resistance). Whole genome transfer will be undertaken in response to a direct request from the commercial breeders. The rationale behind this is, that although targeted

transfer for a specific trait is very valuable, it is recognised that the alien chromatin will also carry genetic variation for a range of target traits, many not currently predictable. Thus, one of the priorities of this work will be to provide researchers and the breeding companies with a series of overlapping alien introgressions for use in field trials and trait analysis in multiple environments.

Figure 4

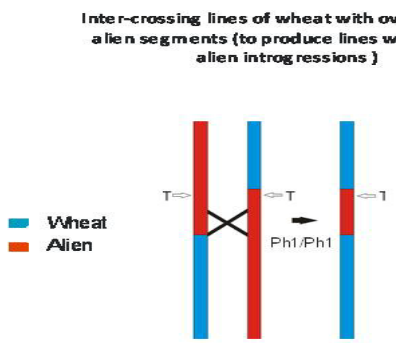
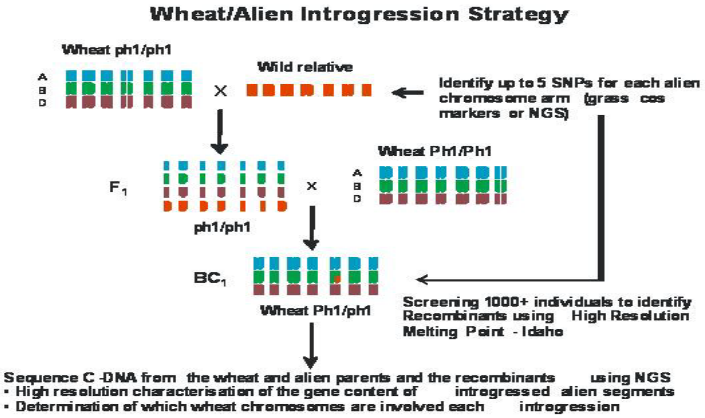


Figure 5



The crossing strategy is shown in Figure 5. This strategy will rapidly yield genome wide introgressions in the BC_1 in only two generations. Wheat/alien introgression results from recombination between the chromosomes of wheat and the alien species at meiosis. However, normal wheat carries *Ph1* (located on the long arm of chromosome 5B), which restricts recombination to homologous (identical) chromosomes, thus preventing recombination between related (homoeologous) wheat and alien chromosomes. In order to induce wheat/alien homoeologous recombination we will initially cross the four alien species to *ph1* mutant lines, i.e. lines that lack the *Ph1* locus. Thus, as a result homoloeogous recombination will occur at meiosis in the F_1 (Figure 5). We will use a *ph1* line in the background of Paragon. Paragon (a modern variety and suited to trait analysis) is a new *ph1* mutant which is widely crossable to a range of alien species (significant seed set has been obtained in pilot experiments with the Paragon *ph1* mutant and *Ae. speltoides*, *T. urartu*, *Th. bessarabicum*, *Secale segetale*, *S. iranicum* and *S. anatolicum*).

The four inter-specific F_1s will be crossed with normal Paragon which carries the wild type *Ph1* locus to generate BC_1 populations that will contain wheat/alien introgressions. About 1,000 BC_1 progeny will be generated from each of the four wheat/alien F_1 populations, i.e. 4,000 progeny in total. Identification of introgressed alien chromosome segments will be achieved primarily using DArT markers, which enable the detection of sequences from the alien species in a wheat background. DArT marker libraries, in excess of 1,000 markers for each of the four alien species, will be generated by Dr Andrzej Kilian (Diversity Arrays). The 1,000 individuals in each of the four BC_1 populations will be screened with the relevant DArT marker library, i.e. the library generated from the alien species that each BC_1 was initially derived. We have previously demonstrated the feasibility of DArT for the detection of introgressions in the *Lolium/Festuca* system. Additional screening will be performed using SNPs that show polymorphism between the alien species and wheat, and genomic *in situ* hybridisation (GISH). The SNPs that will be used are derived from more than 1,000 cross species (COS) markers that we have developed in our work with grass [12]. The physical, chromosomal and/or genetic location of these markers is known in grass, rice, *Brachypodium* and many of the Triticeae, e.g. barley and wheat. In addition, we have shown that a significant proportion (~45%) of the primers developed amplify DNA and show SNPs between wheat (Paragon) and its wild relatives (*T. urartu, Th. bessarabicum*, rye and *Ae. speltoides*). This work was performed using high resolution melting point technology. In addition to these SNPs, a further source of COS markers developed by Simon Griffiths will also be exploited in this programme.

Marker profiles will be used to select specific introgressions for GISH analysis in the BC_1s derived from *Th. bessarabicum* and rye (the potential for using FISH and species specific probes will be explored for *Ae. speltoides* and *T. urartu*). This will 1) confirm the presence of an introgression, 2)

reveal the physical size of an introgression and 3) determine the position of recombinant events. This will enable comparisons to be made between marker profiles and the size of alien segments introgressed and also to determine the presence/absence of the physical clustering of wheat/alien recombinant events.

The combination of DArT marker, SNP and GISH will lead to the rapid detection of most, if not all, alien introgressions generated in the BC_1 population, detecting both single and double wheat/alien recombinants in a chromosome arm. Once identified, 140 selected introgressions derived from each of the four wheat/alien BC_1 populations (140 x 4 = 560) will be characterised at high resolution by Illumina-based genotyping. This will enable 1) an accurate determination of the size (based on synteny with rice and *Brachypodium*) of each alien chromosome segment and 2) identification of lines carrying overlapping chromosome segments.

1.1 High resolution characterisation: Normalised cDNA libraries will be generated from the F_1, Paragon and the alien species and sequenced through the Genotyping cross linking theme. Analysis of Paragon and the alien species will enable the identification of 10,000s SNPs between wheat and the wild species. This will be followed by the sequencing of genomic DNA captured via SureSelect technology from the 140 selected introgressions (two runs of an Illumina sequencer, i.e. selected DNA from 10 [barcoded] introgressions will be genotyped in each of the 7 lanes used in a single Illumina run = 70 introgressions genotyped [this will give 200x coverage of the selected DNA from each introgression line]) for the presence and absence of alien specific SNPs. Characterisation of the size of each introgression (based on approximate gene content) will be achieved via determination of the number of alien SNPs present or absent and comparisons with the alien parent. A similar procedure is presently being used at Bristol to bin map 10,000s of SNPs to wheat deletion bins. Once characterised, each introgression line will be backcrossed once more to wheat to remove the *ph1* deletion and then self-fertilised to produce plants homozygous for the alien introgression. Introgression homozygotes and the genomic insertion point of alien chromosome segments will be determined using wheat genome specific SNPs being developed in the Genotyping cross linking theme. The absence of orthologous wheat SNPs will identify the presence of homozygous introgressions and the genomic region of wheat involved. These plants will be self-fertilised to bulk up seed that will be provided to breeders and researchers for trait analysis during the third year of the project. When a specific region of the alien genome is identified as carrying a specific target gene, the overlapping introgressions for that region will be identified and inter-crossed. However, this work will fall outside the time frame of this project. As a result, small introgressions carrying a target gene, but lacking deleterious genes will be identified.

2. Targeted introgression: A new series of wheat/alien amphidiploids will be generated in the Paragon background, i.e. alien species will be crossed to Paragon and then the resulting hybrids will be chromosome doubled to form amphidiploids. The amphidiploids will be generated from a wide range of different species (several amphidiploids will be generated for each species from several different accessions). Seed of all the parental alien species will be retained and following trait analysis specific alien species will be targeted as the basis of future wheat/alien introgression programmes but with emphasis on a specific region of the alien genome which carries a gene for a target trait, i.e. wheat/alien recombinants will be generated, screened for the target trait and the genomic region which carries the gene responsible identified. This will be followed by the development of overlapping segments for the region of the genome identified, followed by inter-crossing and further trait analysis.

3. Key Target traits*:* The work described above in sections 1 and 2 will result in the development of an extensive wheat/alien germplasm resource, a primary deliverable of the research described. While genetic variation will exist for many target traits in this proposal, we will concentrate on those traits described in the Phenotyping cross linking theme and in addition, in collaboration with collaborators in Australia, Oxford and Saudi Arabia, tolerance to drought, heat, salt and general disease resistance. Initial screening will be made during year two of the project on the amphidiploids described in section 2 and also the four new amphiploids that will be produced using the four alien parents described in section 1. The recombinant lines generated in section 1 will become available for analysis during year three of the programme. In addition, during year one

RRes will screen a range of alien species and accessions for traits such as resistance to Take-All and insect resistance. If important sources of resistance are identified in an alien species, not initially included in the alien programme, they will be introduced for targeted introgression, in the first instance, during year two of the programme

Summary of Outputs:

1. Four genome wide overlapping wheat/alien introgression series
2. Strategies to exploit NGS and other new technologies to transfer small alien chromosome segments quickly and efficiently.
3. A new extensive wheat/alien amphidiploids series that will form a large resource of genetic variation for future wheat improvement
4. Initial exploitation of the lines described above resulting in the development of lines of wheat that: are tolerant to biotic and abiotic stresses, and carry key yield potential traits.

Cross-linking theme- Genotyping

Overall Aim: Use recently developed SNPs to genotype lines and populations generated and used in each of the four pillars and link to the information generated within the phenotyping theme.

Background: SNPs within genes are the marker of choice. In wheat, the task of identifying and using SNPs is complicated by the presence of the three genomes, with the result that most SNPs are intra- and not inter-varietal and represent sequence variants between homoeologs and not homologs from different varieties. This difficulty in detecting varietal SNPs has been a significant block in the use of SNPs for mapping important genes and association genetics-based studies. In wheat, to identify varietal SNPs, to understand the factors affecting their frequency and distribution and to use them for large-scale genotyping, Bristol has used bioinformatics and next generation sequencing (NGS) to identify SNPs from the EST databases and various wheat varieties. For instance, we have utilised "AutoSNP" to screen the databases to identify 3,558 varietal SNPs. In addition, via the Bristol coordinated BBSRC-funded project "Mining the allohexaploid wheat genome for useful sequence polymorphisms" we aim to generate up to 110,000 SNPs in four UK varieties by 2011. Of these, 2,000 will be characterised on 20 wheat lines and 104 will be mapped in the Avalon x Cadenza or Rialto x Savannah DH mapping populations. With the advent of NGS, our ability to identify varietal SNPs in wheat and related (alien) lines has increased to the point where the availability of SNPs will soon no longer be a factor in the high throughput screening of germplasm. However, even though the availability of SNPs will soon no longer be a barrier, the genotyping platforms for their widespread use are still limited. Therefore, in addition to our work in developing SNPs, in this section of the programme, Bristol will establish technologies to genotype materials generated in the four pillars.

Research programme:

1. Collate existing wheat SNPs within a public web based SNP database (1-6 months): Using bioinformatics and NGS, we have identified 14,000 putative wheat SNPs including 8,000 in UK-based varieties. In addition, via the BBSRC-funded project "Mining the allohexaploid wheat genome for useful sequence polymorphisms" we will take this total to 110,000 SNPs. Hence, in the initial phase of the programme, we will collate these SNP into one web-based database. In addition, we have established informal collaborations with Eduard Akhunov and Peter Langridge (see letters of support). As part of this collaboration, Eduard has already supplied the Bristol group with 96 SNPs designed to work on the Illumina Golden Gate platform and we intend to work closely with his group to establish further genotyping technologies. Under the supervision of Dr Gary Barker, an appointed post-doc will collate the available SNPs together with the associated genotyping data into a SNP-database. This database will be made available to the community via the open-access Monogram web site.

2. Generate new wheat SNPs from both diploid, tetraploid and hexaploid *Triticeae* species (1-9 months): Our current SNP's are based on the UK varieties Avalon, Cadenza, Rialto and Savannah and contains little novel germplasm. To correct this, the Bristol group has recently sequenced normalised libraries from two synthetic lines and five lines from the Watkins collection.

This data suggests that the usefulness of any SNP is dependent on the population/source material. In this proposal, we will extend the sequencing of UK lines by sequencing normalised libraries from 24 lines selected to represent a diverse set of UK germplasm, for instance the remaining parents of the MAGIC population (eight for the elite populations and sixteen lines for the diverse populations). In addition, we will sequence the ten Watkins lines used by Simon Griffiths at the JIC to create the Paragon-based SSD populations lines (pillar 1),and up to ten more synthetic lines, including the recently produced synthetic lines from NIAB (pillar 2) together with several examples of *T. urartu, Th. bessarabicum*, *Ae. speltoides* and *Secale* (pillar 3). For each of the normalised libraries (prepared from whole seedlings), we will generate a single Illumina GA2 channel of paired end sequence data equating to approximately 20 million sequences of 2x 100 bases.

3. Generate extremely high-density SNP-based wheat maps (1-18 months): We will use NGS in association with genome selection-based genotyping to generate next generation wheat maps consisting of tens of thousands of SNPs markers. We will do this as follows; SNPs between Avalon, Cadenza and Rialto, Savannah will be synthesised as 120 mer SureSelect probes by Agilent such that the SNPs are present at position 60 within the probe. These will be used to select the appropriate sequences in the 206 lines that make up the Avalon Cadenza mapping population and the 136 lines that make up the Rialto Savannah population. The selected DNA will be subjected to NGS as ten-fold barcoded multiplexed pools (2 million sequences per line) and the sequences analysed for the presence of one or other parental SNP. Genotyping data will be correlated with the available data for the two populations and SNPs assigned to bins as required. This sequencing-based approached to genotyping is, we believe, a step change in genotyping technology, as it does away with the issues surrounding the problems associated with polyploidy and the problems associated with discriminating between varietal and homoeologous SNPs. To confirm the validity of this approach, the Bristol group in collaboration with Professor Neil Hall and Dr Anthony Hall at Liverpool University has already carried out preliminary experiments in which we used a modified version of AutoSNP to generate 3,558 120-mer oligonucleotides. The 3,558 120-mer biotinylated probes were hybridised to size fractioned wheat genomic DNA from Chinese Spring and Cadenza. In small scale 454-based NGS we were able to generate 65,466 sequences from Chinese Spring and 34,114 Cadenza sequences. When aligned to the 3,558 120-mer oligonucleotide probes, 56,196 (86%) of the CS sequences and 29,394 (86%) showed significant sequence similarity (at 1e -10), confirming the procedure was efficient at enriching for specific regions of the wheat genome. Of the original 3,558 probes, for both Chinese Spring and Cadenza, we were able to identify sequences showing homology to 96% of the probes (3,423 for Chinese Spring and 3,410 for Cadenza) showing that in the majority of cases, the probes could select virtually any sequence from the wheat genome. Our results also confirm that ~15% of the SNPs were polymorphic between Chinese Spring and Cadenza a figure in general agreement with wheat SNP PIC values.

4. Establish low, medium and high-throughput genotyping platforms to genotype the various wheat lines generated in the four pillars (6-36 months) SNPs are the most common genetic variation between individuals of the same species and it is therefore not surprising that developing technology for SNP-based genotyping has been the subject of intense activity, with the result that several different platforms now exist. These genotyping platforms have invariably been developed for either human genotyping or for species with defined diploid genomes for which numerous SNPs have previously been developed, for example barley. However, such platforms are of limited use for genotyping polyploids because the presence of highly related, but non-allelic homoeologous sequences complicates the genotyping process. Most systems are incapable of discriminating between highly related, but distinct sequences and so are unable to differentiate between inter or intra-genomic allele variation. Therefore, in polyploid species, it is difficult to determine: a) the allelic content of many of the individual homoeologs within the genome, and b) the contribution that a specific homoeologous allele makes to the breeding material. In this part of the work plan we intend to address this issue and in doing establish the parameters for successful large-scale high-through put wheat genotyping. To carry out this part of the project and in recognition of the requirements of the various commercial and academic end users, we will split our efforts into three approaches: **A.** Small numbers of targeted SNPs with medium to large

numbers of plants. **B.** Medium numbers of SNPs with medium to large numbers of plants and **C.** Large numbers of SNPs with small or medium numbers of plants

A. Small numbers of targeted SNPs with medium to large numbers of plants: Many laboratories, including commercial labs, regularly use small numbers of SNPs to screen thousands of plants. In discussions with the consortium and our industrial partners it is clear there is a need for ~2,100 SNPs (~100 per homoeologous pair), with high PIC values. Fortunately, our current Bristol coordinated BBSRC-funded project "Mining the allohexaploid wheat genome for useful sequence polymorphisms" aims to both generate up to 110,000 SNPs and characterise ~2,000 SNPs on 20 wheat lines, Hence, we believe that at the start of this programme, we will have sufficient well characterised SNPs to genotype large numbers of plants with small numbers of SNPs via or existing SNUPE or SNaPshot technology. Here, we will therefore confirm the utility of this approach by screening materials used or generated by the consortium, for instance, the SSD and CSSL lines generated in pillars 1 and 2, as and when required up to a total of 5,000 plants.

B. Medium numbers of SNPs with medium to large numbers of plants: With the advent of NGS, genotyping is becoming a sequencing-based technology. We are aware of current US funded awards designed to genotype medium numbers of SNPs in significant numbers of plants using Golden Gate technology, however, when applied to medium to large numbers of SNPs this approach is expensive. Hence, rather than employ technologies suggested by Akhunov et al (2009), to genotype the relatively large number of lines produced by the consortium, we will use a highly efficient alternative approach. Here, we will use bar-coded genomic DNA from individual plants in combination with SureSelect genome enrichment to genotype pools of 96 plants in single channels of a GA2 to generate ~20 million sequences. Assuming similar results to our pilot experiments, we expect to generate ~17 million (~86% of 20 million) sequences specific for the probes used. If we employ pools of 5,000 SNP probes, each DNA sample would be represented by 177,000 sequences, which represents a 35 fold over sampling of each SNP probe. We intend to establish three separate assays which will; **1**. Employ 5,000 SNPs characterised in the Watkins collection (pillar 1), **2**. Employ 5,000 SNPs characterised in both selected diploid and tetraploid progenitors and various synthetic lines generated by NIAB (pillar 2) and **3**. Employ 5,000 SNPs characterised in UK material and shown to have relatively high PIC values (pillar 4). Once, developed, the appropriate SureSelect probe pools will be used to genotype the lines generated by the partners in pillars 1,2, 3 and 4 up to a maximum of 6,000 plants.

C. Large numbers of SNPs with small or medium numbers of plants: The ability to do detailed linkage disequilibrium studies in wheat is dependent on population structure and marker density. Population structure is the focus of the work being under taken at NIAB. Here, we will attempt to expand the SureSelect protocol to map at very high density selected lines and populations with the aim of laying the foundation for future work to define genes or areas associated with traits of argonomic importance. To confirm the validity of this approach, we will carry out high-density genotyping of the hexaploid progenitor lines used by the partners, for instance, we will use lines, up to a total of 200, defined by the work at JIC, NIAB and the commercial breeders (pillars 1, 2 and 4). We will use the entire set of SNPs up to a total of 110,000, including sequences defined from exotic material and sources of alien DNA to generate two pools (55,000 each) of SureSelect probes. Following hybridisation SureSelect material will be subjected to NGS to a level of 20-fold redundancy the SNP-based genotype will be automatically called using specific software designed by Bristol to collate polyploid derived SNP data. Information from this ultra high-density genotyping will be analysed in collaboration with JIC, NIAB and IBER to examine the extent of linkage disequilibrium throughout the genome and more importantly around key traits such as *rht* and *Ppd*.

5. Generate a comprehensive database relating SNP polymorphisms with genotype and phenotype (1-36 months in collaboration with JIC, NIAB, Nottingham and RRes): The overall aim of this theme is to establish high throughput genotyping in wheat. However, such data is of little consequence without associating it to phenotype. Throughout the 3-years (and beyond) we will work with NIAB, RRes, Nottingham and JIC to develop an informatics approach to link the considerable amount of genotyping and phenotypic data generated. This relational database and the associated software will be a significant part of the deliverables from this programme.

Summary of Outputs:

1. Collation of existing wheat SNPs within a public web-based SNP database (1-6 months)
2. Generation of new wheat SNPs from both diploid, tetraploid and hexaploid *Triticeae* species (1-9 months)
3. Generation of extremely high-density SNP-based wheat maps (1-18 months)
4. Establishment and use of low, medium and high throughput genotyping platforms to genotype the wheat lines generated in the four pillars (6-36 months)
5. Generation of a comprehensive database relating SNP polymorphisms with genotype and phenotype (1-36 months in collaboration with JIC, NIAB, Nottingham and RRes)

Cross-linking theme- Phenotyping

Overall aim: To identify new phenotypic variation for key UK wheat breeding traits (increased biomass, enhanced N and P use efficiency, and resistance to aphids, bulb fly and Take-All), and to identify the genetic and physiological basis of this variation by screening diverse wheat germplasm collections and precise genetic stocks derived from these collections.

Background: The chosen traits have been selected in discussion with the breeders based on their strategic importance to UK wheat production. In the case of resistance, it is also based on the lack of defined sources of resistance for exploitation in plant breeding. The strategy within this theme will be to screen donor lines, germplasm collections and populations which have been or are being developed in the project, and to provide information on key phenotypes and contributing traits which will inform on crosses to be made in the germplasm development Pillars (1-3) in subsequent years.

Total biomass and nutrient use efficiency: N is a major driver for biomass production; however N fertiliser is expensive and has a large environmental footprint. Wheat crops with low N-uptake and N-use efficiency (grain yield / N available) are associated with nitrate leaching polluting groundwater and global warming, due to emissions of nitrous oxide derived from denitrification of nitrate by soil bacteria. However, optimised yields are required for food security. As a consequence, improving NUE (nitrogen use efficiency) is a key agronomic target at the level of acquisition and in terms of biomass production per unit of N, and both are traits targeted in this proposal. Increasing canopy photosynthetic capacity is a widely recognised pre-requisite to further increase total biomass and NUE in wheat. P is also essential for crop production and is a resource with a finite availability. P fertiliser in the UK is used to maintain optimum soil P-availability, however, in many areas of the world, crop production is at sub-optimum P-availability. Acquisition is the key P-trait for selection, and will be influenced by root architecture, root function (including P-transport and ability to release exudates to enhance bio-availability) and ability to form mycorrhizal associations.

We therefore hypothesise that a range of useable and quantifiable variation exists in yield potential, photosynthetic capacity, N use efficiency and N and P uptake efficiency in the wheat material being generated in this project and furthermore that variation in these complex processes can be resolved into defined heritable traits suitable for genetic analysis which will inform on underpinning mechanisms of nutrient use efficiency and can be utilised for crop improvement.

Aphids: The grain aphid, *Sitobion avenae* and the bird-cherry oat aphid, *Rhopalosiphum padi*, are major pests of wheat, vectoring virulent strains of Barley Yellow Dwarf Virus (BYDV), one of the most damaging cereal viruses in the world. Seed treatment can protect the crop during early growth stages, but an additional application of insecticide may be necessary to control secondary spread from initial colonies. Increasingly stringent regulatory requirements for insecticides will limit the number of such products available to growers. Breeding of resistant varieties has been particularly important in the management of other species of aphids attacking wheat worldwide e.g. the greenbug, *Schizaphis graminum,* and the Russian wheat aphid, *Diuraphis noxia.* However, despite considerable research effort, to date there are currently no commercial wheat varieties resistant to UK aphid species. Recently, in studies in a BBSRC CSI project (BBE0068411) and in WGIN, some evidence of partial resistance has been found to both *S. avenae* and *R. padi* in the

diploid wheat lines, *Aegilops speltoides* (which can have high levels of benzoxazinones (hydroxamic acids) conferring aphid resistance) and *Triticum monococcum*, and in a JIC mapping population from a cross of two hexaploid wheat varieties, Spark and Rialto [13]. The unique resources available through the LOLA project provide a great opportunity for further targeted research on suitable traits for resistance to UK cereal aphid species. Lines to be tested will therefore incorporate *T. monococcum* and *A. speltoides*.

Wheat bulb fly (WBF:*Delia coarctata*): WBF is an important pest of winter wheat in the eastern half of Britain. Seed treatment can be effective for late-sown crops (November onwards) but is not sufficiently persistent to protect crops sown earlier. Post-emergence control relies on organophosphate insecticides (Chorpyrifos and Dimethoate), which could be withdrawn due to the various EU Environment Directives. There has been little research on host plant resistance for this insect. However, some evidence of variation in susceptibility to attack has been shown in lines of *Triticum monococcum* (J Gatehouse, personal communication). This project will identify any resistant material that could be used immediately by UK commercial wheat breeders and any resistant germplasm from more diverse sources for future investigation.

Take All: *(Gaeumannomyces graminis var. triciti, (*Ggt*)*: Globally, Take-All causes the most damaging root disease of wheat. At anthesis, infected crops exhibit patchy stunted plant growth and subsequently premature plant ripening occurs with minimal/no grain fill. The disease symptoms are rarely observed in 1st wheat crops, but are frequently present in 2nd and 3rd wheat crops. Overall losses are estimated to cost £40-60 million annually in the UK alone. There is no known varietal resistance to the disease in commercially cultivated wheat lines and as a consequence no rating is given in either the annual national or recommended listed cultivars. Cultivated wheat is considered to be fully susceptible to Take-All. However, other cereals and grasses have different levels of tolerance to the disease. For example oats is almost immune. Recently some promising sources of resistance have been identified in *Triticum monococcum* (*AA* genome) [14] and certain *Aegilops spp* (*DD* genome). Also other unpublished results from RRes indicates that some hexaploid and tetraploid *Triticum* genotypes exhibit a level of resistance equivalent to that observed in Triticale (Gutteridge, unpublished). Overall, this new data suggests that an exploration of a collection of pre-breeding lines and alien introgression lines, primarily *Ae. tauschii* synthetics from CIMMYT and the best hexaploid / tetraploid material so far identified at RRes is worthy to phenotype in detail for potential resistance to Take-All infection. The overall aim in this project is to identify, within a three year period, the best resistant material that could be immediately deployed by the UK commercial wheat breeders to begin to provide a genetic solution to this severe disease problem. This resistant germplasm from diverse sources would become the core of a future project exploring the underlying mechanisms.

Research Programme

1. Total Biomass and Nutrient use efficiency

1.1 Detailed phenotyping for biomass, NUE and nutrient uptake at high/low N for donor/existing germplasm from the three Pillars will investigate component traits and determine the baseline variation. This will include the scoring of tillering, canopy architectural traits, dry matter and N partitioning, photosynthetic capacity and efficiency at anthesis, grain and straw yields, and N and P accumulation (and other elemental analysis) at harvest. The number of donor lines is expected to be around 230 lines in year 1 and 2. Donor lines (Watkins lines, Gediflux lines, synthetics etc) will be assessed at 50 and 200 kgN/ha in replicated trials at both Rothamsted and Nottingham (in years 1 and 2), and for selected lines, at high and low P (Rothamsted Sawyers long term variable P site in replicated trials in years 2 and 3), to encompass genetic, site and year to year variation. Trials will be in microplots (approx 1 m x 1 m), however head rows or tussocks will be used in case of restricted seed availability. Expertise of the breeding community will be used to assist in setting up trials.

1.2 High throughout phenotyping/screening of germplasm including existing segregating populations to enable physiological dissection and gene discovery (QTL) for target traits (up to 1,000/2,000 lines anticipated in years 2-3, respectively) will be carried out at high/low N. Selected lines will be scored for genetic variation in canopy architectural traits, photosynthetic capacity and efficiency, yield potential, harvest index, N and P acquisition and partitioning. Most individual lines

(e.g. SSD, DH, synthetics) will be screened at one site only (either Rothamsted or Nottingham) at high/low N in replicated trials in years 2 and 3 due to the large amount of plant material involved. Germplasm will be assigned to either Rothamsted or Nottingham for assessment, and in each case lines will be spread over 2 years as determined by availability and capacity. At each site up to 700 lines in year 2 and 1100 lines in year 3 will be screened.

1.3 Selected germplasm showing wide variation in subtraits contributing to the major targets will receive more detailed analysis. For example in years two and three this will include isogenic pairs of lines extracted from F_4 or F_5 Watkins x Paragon SSD lines that were heterozygous at QTL identified as influencing the trait of interest. This will allow a more precise description of how the genetic variation identified exerts an effect on the trait of interest. Where the gene discovery vehicle was a doubled haploid (DH) population, lines will be chosen based on uniformity of genetic background outside of the QTL of interest. Again this allows deeper phenotypic and agronomic characterisation of specific genes. By the end of year 3 NILs in UK adapted parents will be available from the germplasm development Pillars. These will be ideal materials for assessing the impact of the new phenotypic variation for UK breeding and agriculture.

1.4 Selected lines will also be analysed for P-acquisition broken down into analysis of multiple root characteristics involved in P-acquisition. 20 lines will be screened at high and low P in year 2 and 3 for P-yield responses and additional lines from all germplasm pools will be screened for overall P-acquisition efficiency (all years).

1.5 Final grain and straw yields will be assessed for all germplasm to assess biomass accumulation, partitioning and total nutrient uptake efficiency. Full N and elemental (including P) analyses will be carried out for donor lines, with utilization of NIRS for high throughput N analyses, including development of calibrations, for pre-breeding germplasm. Mineral nutrient acquisition will be assessed by tissue ICP analysis for donor material and selected pre-breeding lines.

1.6 Sub-sets of lines (> 50 lines) will be examined using advanced phenotyping to understand the mechanisms determining genetic variation in biomass and NUE (year 1). These measurements will include canopy assessments: developmental (visual score), canopy LAI (Sunseeker), NVDI (Crop Circle), height, senescence (visual/spad), leaf N (spad), photosynthetic capacities and efficiencies using Licor 6400 and chlorophyll fluorescence (capacity for carbon assimilation, stomatal conductance, CO_2 compensation point (where feasible), in situ quantum yield of PSII, Fv'/Fm'), and temperature (IR thermometer).

1.7 Root function will be screened as total nutrient taken up (at high and low N and variable P availability), and best lines will be scored for root and root hair proliferation, as well as exudate production and mycorrhizal association with quantification methods utilising appropriate PCR based assays (all years).

Complementarity between trials at Rothamsted and Nottingham*:*

Trials at two contrasting sites will allow G x E factors to be considered, will provide the required replication of trials within a 3 year time-frame and importantly will facilitate the characterisation of the large amounts of pre-breeding germplasm generated in the project at high/ low N in replicated trials in years 2 and 3. Core phenotyping (yield and N) will be standardised at both sites, however detailed physiological phenotyping will be co-ordinated between sites, exploiting differential expertise of the two teams.

2. Pests and Pathogens

Aphids: Clones of *S. avenae* and *R. padi*, originating from individuals recently collected from the field, will be reared on wheat seedlings under controlled environment conditions. In replicated assays, individual alate aphids will be confined to single seedlings of wheat test lines and to seedlings of the wheat variety Solstice and allowed to produce nymphs for 24-48h, after which the number of nymphs produced on each seedling will be recorded. Since all lines cannot be tested altogether, a double replication of Solstice will be included in each trial to provide a comparative standard. The number of nymphs produced on the test varieties will be expressed as a proportion of the number of nymphs on Solstice in that trial to build a "preference index" that can be used to compare results between trials.

Wheat bulb Fly (*Delia coarctata*): In each of the three years, a selection of wheat lines, including some designated standard varieties, will be grown by JIC in replicated field plots at Church Farm

nr. Norwich (this site is selected as there is likely to be a good infestation of wheat bulb fly). In early spring, replicated short sections of rows of plants will be collected from each plot and kept under cool conditions until assessed. Plants will be scored for symptoms of 'deadheart' and tillers will be dissected and wheat bulb fly larvae or evidence of damage.

Take-All: We will not undertake a systematic screening of all sources of Take-All resistance which is the subject of a community-based proposal currently being developed. However, the donor lines used and some of the pre-breeding lines developed in this programme will be screened for resistance along with the most promising landrace materials so far identified within the WGIN project.

Phenotyping: Phenotyping will be carried out using an Alpha design field experiments and a plot size of 50 cm^2 containing 3 rows each sown with 15 seeds and a 3rd wheat high disease situation. Suitable resistant control species, oats, rye, triticale and *T. monococcum* and fully susceptible hexaploid elite wheats will be present within the trial in blocks and single plots to benchmark the test genotypes. This approach, developed within WGIN, provides a high-throughput screening platform specifically for Take-All [15]. The plant roots systems from each accession will be sampled in July (GS71) and then assessed for root numbers as well as Take-All incidence and severity. After the end of year 2, and with addition summer help, the root systems will be assessed immediately and the 2 full years of data statistically analysed to make the < 200 line selection for further field testing. In year 3 the Alpha design will be sown twice to provide two independent data sets per line.

1. Detailed field phenotyping of 180 potential donor lines identified in the Watkins / Improved Gediflux collections will be carried out in years 1 and 2 in 5 replicate plots in a 3rd wheat high disease pressure environment. The best 30 lines will be tested with 10 replicate plots in year 3.
2. Detailed primary phenotyping of 250 *Ae. tauschii* donor lines for synthetics and 250 alien donor lines for introgression, will be carried out in years 1 and 2, with the number being reduced to about 200 in total in year 3.
3. Detailed field phenotyping of 40 new synthetics will be carried out in years 2 and 3 with 5 replicate plots in a 3rd wheat high disease pressure environment.
4. Take-All pot tests in controlled environment rooms will be carried out to determine which germplasm sources also provide root protection at the seedling stage These will be done on the 180 lines from activity 1 in year 1 and on about 200 lines from activities 2 and 3 in year 3.

Replicated 5 week duration pot tests will be carried out under controlled environment conditions by sowing seed using a mixture of 6 characterised Ggt isolates added into 'naïve' soil (10 seed s/pot and 5 pots for each accession in each test. These disease assessments will be aided by the use of a low power binocular microscopy of either stained or unstained roots. Follow up pot tests of 1 to 3 weeks duration will be done on the top 20% of resistant material identified to determine the initial root response, surface runner hyphae formation, the extent of penetration, and whether an overall root architecture change occurs in response to infection (for example secondary rooting). Ggt fungal biomass will be quantified using RT-PCR. Maintenance of root function will be explored by dye uptake experiments. For the top 5% of sources of resistance identified SEM analysis of the roots will be done. These experiments will permit the most accurate, robust and high throughput screening protocols to be developed for the identification and quantification of resistance. This detailed phenotype knowledge will aid their future genetic characterisation.

Phenotypes screened in addition to those within programme: The alien introgression germplasm will be screened for tolerance to drought in a new field facility being developed in Australia (Professors Peter Sharp and Richard Trethowan, University of Sydney). This work will be extended to screening for stem, stripe and yellow rust and Fusarium Crown rot. Salt and heat tolerance testing will be performed in collaboration with Professor Nick Harberd (Oxford University) in Saudi Arabia (Caust University). In addition to the material described lines of wheat which carry a single *Th. bessarabicum* chromosome which confers salt tolerance will also be exploited. Targeted introgression is expected to yield salt tolerant germplasm which carries a single small introgression by the end of the project (funding for this will be provided by Professor Harberd's group at Oxford).

Summary of Outputs The screening to be undertaken on donor lines (existing material) and 'pre-breeding lines' (generated material, crosses, SSDs, NILs, synthetics):

NUE/Biomass

- Donor lines: 230 in years 1-2 (in both RRes and Nottingham)
- Prebreeding lines: >1,000 in year 2, >2,000 in year 3 (shared RRes and Nottingham)

P acquisition

- Donor lines: 20 in years 1-3 (RRes)

Aphids

- Donor lines: 1,000 in year 1
- Prebreeding lines: 1,000 in years 2 and 3

Bulb fly

- Donor lines: 500 in year 1
- Prebreeding lines: 500 in years 2 and 3

Take All

- Donor lines: 500 in years 1 and 2, 200 in year 3
- Gediflux/Watkins lines: 200 in years 1, 2, 30 in year 3
- Prebreeding lines: 40 in years 2 and 3

Collaboration with international wheat improvement efforts: To maximise the benefits to UK wheat breeding and to global food security it is essential that synergistic international interactions are established. A member of CIMMYT will be part of the Steering committee. Letters of support for this proposal have been provided by the international wheat community. Seed samples will be made available to the International Wheat Improvement Network of the CGIAR system (CIMMYT and ICARDA). At the National level pre-breeding germplasm exchanges will be made for example with the Australian Grains Research and Development Council (GRDC), the Chinese Academy of Agricultural Sciences, and the Indian Council of Agricultural Research. A number of important traits will not be pursued within this programme. Groups working in these areas will have free access to germplasm collections, mapping populations, and molecular marker information.

Funding the programme after the LOLA finishes: We will achieve significant progress in the generation of novel germplasm and preliminary characterisation of lines within the scope of the three year research programme. However, the full impact in terms of delivery requires a longer timeframe than a three year grant. This LOLA will provide funding to pump prime this area on a sufficient scale to generate novel germplasm within a practicable timescale. We are actively seeking further funding from other avenues to cover the programme beyond that funded through the LOLA.

Concluding remarks: This proposal aims to initiate the development of novel key wheat germplasm as a foundation for exploitation in wheat breeding programmes and academic research, both in the UK and internationally, in years to come. The germplasm will be used to dissect key traits in wheat and will form the basis of future biological programmes to understand the molecular basis of these traits. The availability of such resources will attract the next generation of UK researchers to study wheat and provides the basis for the UK to contribute to addressing the growing international problem of Food Security in the coming years.

References

[1] www.foodsecurity.ac.uk; [2] www.jic.ac.uk/summerprogramme/; [3] van Ginkel M., Ogbonnaya F. (2005) Field Crops Research **104**: 86-94; [4] Uauy C., *et al.* (2005) Theoretical and Applied Genetics **112**: 97-105; [5] Uauy C., *et al.* (2006) Science **314**:1298-301; [6] Reynolds M., *et al.* (2009) *Journal of Experimental Botany* **60**:1899-1918; [7] Fridman E., *et al.* (2004) Science **305** : 1786; [8] Kubo T., *et al.* (2002) Breeding Science **52** : 319-325 ; [9] Sears, E.R. (1956) Brookhaven Symp. Biol. **9**:1-22; [10] Sears, E.R. (1972) Stadler Symp. **4**:23-38; [11] McIntosh, R.A. (1983) 6th Int. Wheat Genet. Symp. 777-783 [12] King J., *et al*. (2007) *Genetics* **177:** 597-606;
[13]www.wgin.org.uk/wgin_2003-2008/Stakeholders/WGINStakeholderNewsletterOctober2009.pdf;
[14]www.wgin.org.uk/wgin_2003-2008/Stakeholders/WGINStakeholderNewsletterOctober2007.pdf;
[15] www.wgin.org.uk/wgin_2003-2008/Stakeholders/WGINStakeholderNewsletterOctober2008.pdf

Work Programme Time table

	2010				2011											
	September	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December
Landrace pillar	Sow 1000 Watkins lines in 6m^2 plots		Phenotype 1000 Watkins lines from 6m^2 plots									Harvest Watkins from 6m^2 plots; should get 1.2Kg (assumes 1/4 yield)			Phenotype Gediflux from 1m^2 plots	
				Sow 10 Paragon x Watkins SSD-F_5 populations (=960 lines) in glasshouse for DNA extraction			Extract 960 Paragon x Watkins SSD DNA						Sow Gediflux & 1000 varieties in 1m^2 plots			
											Molecular markers for Paragon x Watkins SSD populations					
	Sow Paragon x Watkins F_5 ear rows to multiply and phenotype		Phenotype F_5 ear rows										Sow Paragon x Watkins bulked F_6 in 1m^2 plots		Phenotype bulked F_6	
									Paragon x Watkins QTL analysis							
															Make F_1s for Paragon x Watkins NILs	
	Database development															
										Summer school						
Synthetics pillar	Select diverse accessions of diploid and tetraploid donor plants												Develop single chromosome founder lines from *Ae tauschii* & emmer wheat for future production of CSSL populations as resources for QTL mapping & positional cloning			
	Produce pentaploid F_1 lines by hexaploid x tetraploid hybridization and novel synthetic hexaploid wheat by tetraploid x diploid hybridization															
	In Paragon & Robigus produce 1600-2000 BC_1F_5 lines from 20-25 cultivated emmer & durum donors and 400 BC_1F_5 lines from pre-project SHW; initiate development of equivalent material from novel synthetic wheat															
	Collect physiological data on phenotypes expected to affect yield in emmer wheat; select genotypes for mapping population and CCSL production												Initiate development of 3 wild emmer mapping populations			
Alien introgression pillar	Genome wide introgression: Production of wheat *ph1ph1* x alien species F_1 hybrids; initial SNP development; isolation of DNA for DArT marker library construction							Genome wide introgression: Backcross F_1 hybrids to Paragon to generate wheat/alien recombinants; production of additional F_1 progeny if required; continued SNP development; continued development & trials of DArT markers						Genome wide introgression: Detection of wheat/alien recombinants; Via DArT, backcross wheat/alien recombinants to wild type Paragon; Initial SNP & GISH characterisation		
	Targeted introgression: Production of wheat/alien amphihaploids							Targeted introgression: Chromosome doubling of amphihaploids to produce amphidiploids; Self-pollination of newly synthesised amphidiploids						Targeted introgression:Seed multiplication; initial trait analysis		
High throughput genotyping	Collate existing wheat SNPs within a public web-based SNP database						Establish and use low, medium and high genotyping platforms to genotype the wheat lines generated in the four pillars									
	Generate new wheat SNPs from both exotic materials (Watkins collection, synthetic, etc) and related species															
	Generate extremely high-density SNP-based wheat maps															
	Generate a comprehensive database relating SNP polymorphisms with genotype and phenotype (in collaboration with JIC, NIAB, Nottingham & Rothamsted)															
Phenotyping	Detailed phenotyping of donor germplasm (~230 lines) for biomass and Nitrogen use efficiency (NUE) (trials at RRes & Nottingham) (Year 1)												Detailed phenotyping of donor germplasm (~230 lines) for biomass and NUE (trials at RRes & Nottingham) (Year 2)			
													High throughout phenotyping/screening of pre-breeding lines (up to 1000/2000 lines anticipated) carried out at high/low N (Year 1)			
	Analysis of selected lines for P-acquisition trait (Year 1)												Analysis of selected lines for P-acquisition trait (Year 2)			
	Rearing of clones of the wheat aphid and bird-cherry oat aphid on test wheat lines to build a "preference index" (500 donor lines) (Year 1)												Rearing of clones of the wheat aphid and bird-cherry oat aphid on test wheat lines to build a "preference index" (1000 donor lines) (Year 2)			
	Scoring of wheat lines for symptoms of "deadheart" and other damage caused by the wheat bulb fly (*Delia coarctata*) (Year 1)												Scoring of wheat lines for symptoms of "deadheart" and other damage caused by the wheat bulb fly (*Delia coarctata*) (Year 2)			
	Detailed primary phenotyping of donor germplasm and prebreeding lines for resistance to the Take all fungus (500 donor/prebreeding lines; 180 Gediflux/Watkins lines) (Year 1)												Detailed primary phenotyping of donor germplasm and prebreeding lines for resistance to the Take all fungus (500 donor/prebreeding lines; 180 Gediflux/Watkins lines) (Year 2)			

Year 1 Year 2

	2012												2013							
	January	February	March	April	May	June	July	August	September	October	November	December	January	February	March	April	May	June	July	August
Landrace pillar	Phenotype Gediflux from 1m^2 plots							Harvest Gediflux from 1m^2 plots					SSD from new crosses (Watkins, Gediflux, 1000)							
									Sow Gediflux & 1000 varieties multiplication in 6m^2 plots		Phenotype Gediflux from 6m^2 plots									Harvest Gediflux from 6m^2 plots
	Molecular markers & linkage maps for Paragon x Watkins SSD populations										Develop informative markers for QTL of interest									
	Phenotype bulked F_6								Sow Paragon x Watkins bulked F_6 in 6m^2 plots		Phenotye bulked F_7									Harvest bulked F_7
	Paragon x Watkins QTL analysis																			
					BC_1 for Paragon x Watkins NILs						BC_2 for Paragon x Watkins						Select BC_2F_2 homozygotes		
												New crosses: Watkins, Gediflux, 1000						New crosses: Watkins, Gediflux, 1000		
	Database development																			
						Summer school												Summer school		
Synthetics pillar	Develop single chromosome founder lines from *Ae tauschii* & emmer wheat for future production of CSSL populations as resources for QTL mapping & positional cloning								Identify and select single chromosome founder lines from *Ae tauschii* & emmer wheat for future production of CSSL populations as resources for QTL mapping & positional cloning											
	Produce pentaploid F_1 lines by hexaploid x tetraploid hybridization and novel synthetic hexaploid wheat by tetraploid x diploid hybridization																			
	In Paragon & Robigus produce 1600-2000 BC_1F_5 lines from 20-25 cultivated emmer & durum donors and 400 BC_1F_5 lines from pre-project SHW; initiate development of equivalent material from novel synthetic wheat																			
	Initiate development of 3 wild emmer mapping populations								Continue development of 3 wild emmer SSD mapping populations											
Alien introgression pillar	Genome wide introgression: Detection of wheat/alien recombinants; Via DArT, backcross wheat/alien recombinants to wild type Paragon; Initial SNP & GISH characterisation				Genome wide introgression: Self fertilisation of wheat/alien recombinants; High resolution characterisation via sequencing of selected recombinants; Continued SNP & GISH analysis					Genome wide introgression: Identification of lines homozygous for wheat/alien recombinants followed by self poliination to initiate seed multiplication; continued characterisation; initial trait analysis						Genome wide introgression; Further seed multiplication; distribution of seed for full trait analysis			
	Targeted introgression:Seed multiplication; initial trait analysis				Targeted introgression: Further seed multiplication, full trait analysis					Targeted introgression: Intercross alien species, identified through trait analysis of amphidiploids, with Paragon *ph1ph1* mutant to produce new F_1 hybrids; continued seed multiplication of all amphidiploids						Targeted introgression: Backcross F_1 hybrids to wild type Paragon			
High throughput genotyping	Establish and use low, medium and high genotyping platforms to genotype the wheat lines generated in the four pillars																			
	Generate extremely high-density SNP-based wheat maps																			
	Generate a comprehensive database relating SNP polymorphisms with genotype and phenotype (in collaboration with JIC, NIAB, Nottingham & Rothamsted)																			
Phenotyping	Detailed phenotyping of donor germplasm (~230 lines) for biomass and Nitrogen use efficiency (NUE) (trials at RRes & Nottingham) (Year 2)																			
	High throughout phenotyping/screening of pre-breeding lines (up to 1000/2000 lines anticipated) carried out at high/low N. Scoring for genetic variation in canopy architectural traits, photosynthetic capacity and efficiency, yield potential, harvest index, N and P acquisition and partitioning (Year 1)								High throughout phenotyping/screening of pre-breeding lines (up to 1000/2000 lines anticipated) carried out at high/low N. Scoring for genetic variation in canopy architectural traits, photosynthetic capacity and efficiency, yield potential, harvest index, N and P acquisition and partitioning (Year 2)										
	Analysis of selected lines for P-acquisition trait (Year 2)								Analysis of selected lines for P-acquisition trait (Year 3)											
	Rearing of clones of the wheat aphid and bird-cherry oat aphid on test wheat lines to build a "preference index" (1000 donor lines) (Year 2)								Rearing of clones of the wheat aphid and bird-cherry oat aphid on test wheat lines to build a "preference index" (1000 donor lines) (Year 3)											
	Scoring of wheat lines for symptoms of "deadheart" and other damage caused by the wheat bulb fly (*Delia coarctata*) (Year 2)								Scoring of wheat lines for symptoms of "deadheart" and other damage caused by the wheat bulb fly (*Delia coarctata*) (Year 3)											
	Detailed primary phenotyping of donor germplasm & prebreeding lines for resistance to 'Take all' (500 donor/prebreeding lines; 180 Gediflux/Watkins lines) (Year 2)								Detailed primary phenotyping of donor germplasm & prebreeding lines for resistance to 'Take all' (200 donor/prebreeding lines; 180 Gediflux/Watkins lines) (Year 3)											

Year 2 Year 3